Molecular Structures of Amino Acids and Peptides.*
III. The Molecular Structure and Conformation of Potassium
L-Tyrosine-*O*-sulfate Dihydrate†
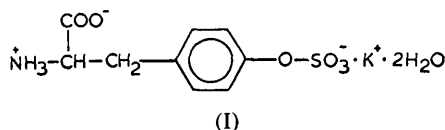
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L-Tyrosine-O-sulfate is a constituent of both human and porcine hormones gastrin and cholecystokinin-pancreozymin. The potassium salt of L-tyrosine-O-sulfate crystallized as a dihydrate in space group $P2_12_12_1$ with $a = 19\cdot221 \pm 0\cdot001$, $b = 11\cdot946 \pm 0\cdot001$ and $c = 5\cdot893 \pm 0\cdot001$ Å. The calculated density for four formula units of $C_9H_{11}NO_6S.2H_2O$ is $1\cdot651$ g.cm^{-3}, measured $1\cdot649$ g.cm^{-3}. The structure was determined by the conventional heavy-atom technique and refined by full-matrix least squares to an R value of $0\cdot039$. The penta-coordinated K$^+$ ion is chelated to the sulfate oxygen atom and the carboxyl oxygen atom of the same molecule. The crystal structure is dominated by ionic bonds and hydrogen bonds. One of the water protons is not involved in hydrogen bonding nor is the sulfate ester oxygen atom. The conformation about the C(2)–C(3) bond is *syn*, rather than *anti* commonly observed in the absence of metal ions. The amino nitrogen atom is $0\cdot4$ Å out of the plane of the carboxylate group. The ester oxygen–sulfur bond makes a projected angle of 56° with the aromatic ring. A conformational analysis of tyrosine derivatives is given; the mean torsional angle about the C(3)–C(4) bonds is about 92° with a range of 51°.

Introduction

L-Tyrosine-*O*-sulfate (I) is a constituent of both human and porcine hormones, gastrin (Gregory, Hardy, Jones, Kenner & Sheppard, 1964; Gregory, Tracy & Grossman, 1966) and cholecystokininpancreozymin (Mutt & Jorpes, 1968), and has also been isolated from bovine fibrinopeptide B (Krajewskii & Blomback, 1968) and fibrinogen.

(I)

Experimental

Potassium L-tyrosine-*O*-sulfate dihydrate crystallizes in the orthorhombic space group $P2_12_12_1$, forming large needle-shaped crystals elongated along the *c* axis. The crystals were kindly supplied to us by Dr Miguel L. Ondetti. The unit-cell dimensions are listed in Table 1. These dimensions were obtained from a least-squares fit of the diffractometer angles χ, φ and 2θ measured for 30 axial and diagonal reflections with 2θ settings greater than 40°, with nickel-filtered copper

radiation ($\lambda = 1\cdot5418$ Å). The density of the crystals was determined by flotation in a solution of bromoform and chloroform and agrees with the calculated density assuming two waters of crystallization per asymmetric unit.

Table 1. *Crystal data for* $H_{11}C_9NO_6S . 2H_2O$

Space group	$P2_12_12_1$
a	$19\cdot221 \pm 0\cdot001$ Å
b	$11\cdot946 \pm 0\cdot001$
c	$5\cdot893 \pm 0\cdot001$
V	1353 Å^3
Z	4
D_{calc}	$1\cdot651$ g.cm^{-3}
D_{obs}	$1\cdot649$ g.cm^{-3}

A $0\cdot11 \times 0\cdot15 \times 0\cdot25$ mm crystal, cut from a $0\cdot60$ mm long needle, was mounted on our Picker diffractometer with its c axis along φ. Altogether 1399 reflections were scanned using the 2θ scan technique with nickel filtered Cu $K\alpha$ radiation ($\lambda = 1\cdot5418$ Å) to a limit of $2\theta = 133\cdot7°$. Of these, 20 reflections were not observed above background. Corrections were applied to the data for Lorentz and polarization effects as well as for background and variation in the standard intensity. No corrections were made for absorption.

Structural solution

The solution of the crystal structure was accomplished using conventional heavy-atom techniques. A Patterson synthesis $P(uvw)$ computed using normalized intensities (E^2) as coefficients yielded the potassium and sulfur atom positions. Three cycles of structure factor and Fourier calculations revealed all of the non-

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hydrogen atoms in the structure with the value of $R=0.21$.

Structural refinement

Refinement was by the method of full-matrix least-squares. After four cycles of isotropic refinement the R index was 0·08 and the non-hydrogen atoms had converged. A difference electron density map revealed the ten hydrogen atoms associated with the amino acid. The peak heights of the hydrogen electron densi-

Table 2. *Atomic coordinates of potassium* L-*tyrosine-O-sulfate dihydrate*

	x	$\sigma(x)*$	y	$\sigma(y)*$	z	$\sigma(z)*$
K	0·4941	5	0·1958	7	0·4485	2
S	0·4766	5	−0·1383	7	0·5565	2
O(1)	0·2788	2	0·3520	3	0·6411	5
O(2)	0·3846	1	0·3433	2	0·4925	5
O(3)	0·4247	1	−0·1307	2	0·3357	5
O(4)	0·4330	2	−0·1769	3	0·7413	5
O(5)	0·5050	2	−0·0286	2	0·5898	7
O(6)	0·5257	1	−0·2227	2	0·4849	6
O(7)	0·6349	2	0·1179	3	0·3560	7
O(8)	0·6091	3	0·0881	4	0·8945	9
N(1)	0·3400	2	0·3842	3	0·0688	7
C(1)	0·3202	2	0·3535	3	0·4807	7
C(2)	0·2869	2	0·3570	3	0·2452	7
C(3)	0·2508	2	0·2452	3	0·1920	8
C(4)	0·2971	2	0·1446	3	0·2296	7
C(5)	0·3443	2	0·1108	3	0·0653	8
C(6)	0·3887	2	0·0200	3	0·1049	8
C(7)	0·3844	2	−0·0361	3	0·3085	8
C(8)	0·3376	2	−0·048	3	0·4719	8
C(9)	0·2995	2	0·0866	3	0·4316	8
H(1)	0·354	2	0·464	3	0·116	7
H(2)	0·376	2	0·338	3	0·061	7
H(3)	0·322	2	0·379	3	−0·083	7
H(4)	0·255	2	0·421	3	0·252	7
H(5)	0·207	2	0·240	3	0·297	7
H(6)	0·233	2	0·252	3	0·054	4
H(7)	0·339	2	0·150	3	−0·079	7
H(8)	0·428	2	−0·005	3	−0·005	7
H(9)	0·332	2	−0·038	3	0·623	7
H(10)	0·264	2	0·113	3	0·539	7
H(11)	0·678	2	0·145	3	0·399	8
H(12)	0·645	2	0·104	4	0·242	8
H(13)	0·601	3	0·001	5	0·851	9
H(14)	0·564	3	0·075	5	1·056	9

* $\sigma \times 10^5$ for non-hydrogen atoms and $\sigma \times 10^3$ for hydrogen atoms.

Table 3. *Anisotropic thermal parameters* $\times 10^5$ *for potassium* L-*tyrosine-O-sulfate dihydrate*

The temperature factor is of the form $T = \exp\left[-(\beta_{11}h^2 + \ldots + 2\beta_{12}hk\ldots)\right]$.

	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
K(1)	258	431	3240	20	−163	−10
S(1)	158	373	2127	10	−34	−7
O(1)	225	868	1676	7	61	1
O(2)	190	716	2309	35	−151	−127
O(3)	232	401	2168	76	−95	−128
O(4)	263	786	2159	47	207	314
O(5)	284	413	4253	−71	−330	60
O(6)	177	546	3469	100	111	27
O(7)	293	666	4278	5	171	118
O(8)	606	846	6068	−94	−612	214
N(1)	163	498	1652	1	24	−17
C(1)	196	366	1554	−8	−61	−122
C(2)	149	350	1746	7	−31	−36
C(3)	156	391	1805	7	−94	65
C(4)	120	357	1929	−20	−97	−214
C(5)	226	399	1690	16	−84	16
C(6)	217	46∩	1839	39	−31	−213
C(7)	146	33(	2020	−9	−95	--74
C(8)	178	510	1955	−5	9	116
C(9)	148	421	2∩53	−6	23	90

Table 4. *Observed and calculated structure factors* × 10

Reflections marked with asterisks were not used in the refinement since they showed considerable differences between F_o and F_c.

ties were of the order of 0·4–0·6 e.Å⁻³. The four hydrogen atoms associated with the two molecules of water of crystallization could not be located unequivocally. Two cycles of anisotropic refinement on all the non-hydrogen atoms and the hydrogen atom positional parameters reduced R to 0·05. A second difference synthesis revealed the water hydrogen positions. An additional four least-squares cycles varying the positional and anisotropic parameters of the non-hydrogen atoms and the positional parameters of the hydrogen atoms gave a final R of 0·039. The refinement was terminated at this point as the ratios of the shifts to the estimated standard deviations were in general less than 0·1. During the refinement the hydrogen

temperature factors were held fixed, with a B value of 5·5 Å².

The weighting scheme used was based on the counting statistics for a given reflection modified to take into account systematic errors presumed to be due to secondary extinction effects for the strong low-order reflections. The weight for a given reflection was

$$\sigma(F)=\frac{F}{2}\frac{[I+B+0\cdot02\,(I-B)^2]^{1/2}}{(I-B)}+0\cdot02F,$$

where I = scanned intensity, B = total background and F = structure amplitude. The atomic form factors used throughout this analysis were those of Cromer &

Table 5. *Bond lengths in potassium* L-*tyrosine-O-sulfate dihydrate*

E.s.d.'s in parentheses.

i	j	D_{ij}	i	j	D_{ij}
S	O(3)	1·611 (3) Å	O(7)	H(11)	0·91 (4) Å
S	O(4)	1·449 (3)	O(7)	H(12)	0·82 (5)
S	O(5)	1·434 (3)	O(8)	H(13)	0·86 (6)
S	O(6)	1·444 (3)	O(8)	H(14)	1·09 (6)
C(1)	O(1)	1·250 (5)	N(1)	H(1)	1·03 (4)
C(1)	O(2)	1·246 (5)	N(1)	H(2)	0·89 (4)
C(1)	C(2)	1·528 (6)	N(1)	H(3)	0·96 (4)
C(2)	N(1)	1·492 (5)	C(2)	H(4)	0·99 (4)
C(2)	C(3)	1·538 (6)	C(3)	H(5)	1·05 (4)
C(3)	C(4)	1·511 (5)	C(3)	H(6)	0·89 (3)
C(4)	C(5)	1·387 (6)	C(5)	H(7)	0·98 (4)
C(4)	C(9)	1·379 (6)	C(6)	H(8)	1·04 (4)
C(5)	C(6)	1·400 (6)	C(8)	H(9)	0·98 (4)
C(6)	C(7)	1·377 (6)	C(9)	H(10)	0·92 (4)
C(7)	O(3)	1·410 (4)			
C(7)	C(8)	1·370 (6)			
C(8)	C(9)	1·389 (5)			

Table 6. *Bond angles in potassium* L-*tyrosine-O-sulfate dihydrate*

E.s.d.'s in parentheses.

i	j	k	ijk	i	j	k	ijk
S(1)	O(3)	C(7)	118·8 (0·2)°	H(1)	N(1)	C(2)	101·0 (1·5)°
O(3)	S(1)	O(4)	106·6 (0·2)	H(1)	N(1)	H(2)	112·9 (1·8)
O(3)	S(1)	O(5)	106·4 (0·2)	H(1)	N(1)	H(3)	113·6 (1·8)
O(4)	S(1)	O(5)	114·1 (0·2)	H(2)	N(1)	C(2)	115·7 (1·6)
O(3)	S(1)	O(6)	100·8 (0·1)	H(2)	N(1)	H(3)	101·1 (1·8)
O(4)	S(1)	O(6)	112·1 (0·2)	H(3)	N(1)	C(2)	113·1 (1·6)
O(5)	S(1)	O(6)	115·4 (0·2)	H(4)	C(2)	N(1)	106·5 (1·5)
O(1)	C(1)	O(2)	126·4 (0·3)	H(4)	C(4)	C(1)	104·3 (1·6)
O(1)	C(1)	C(2)	115·6 (0·3)	H(4)	C(2)	C(3)	113·8 (1·6)
O(2)	C(1)	C(2)	117·9 (0·3)	H(5)	C(3)	C(2)	107·1 (1·5)
O(3)	C(7)	C(6)	117·0 (0·3)	H(5)	C(3)	C(4)	109·8 (1·5)
O(3)	C(7)	C(8)	121·6 (0·3)	H(5)	C(3)	H(6)	103·8 (1·6)
N(1)	C(2)	C(1)	110·7 (0·3)	H(6)	C(3)	C(2)	106·5 (1·4)
N(1)	C(2)	C(3)	110·9 (0·3)	H(6)	C(3)	C(4)	115·7 (1·4)
C(1)	C(2)	C(3)	110·5 (0·3)	H(7)	C(5)	C(4)	113·4 (1·5)
C(2)	C(3)	C(4)	113·2 (0·3)	H(7)	C(5)	C(6)	125·6 (1·5)
C(3)	C(4)	C(5)	121·0 (0·3)	H(8)	C(6)	C(5)	123·9 (1·5)
C(3)	C(4)	C(9)	120·4 (0·3)	H(8)	C(6)	C(7)	116·9 (1·5)
C(4)	C(5)	C(6)	120·6 (0·3)	H(9)	C(8)	C(7)	126·9 (1·5)
C(4)	C(9)	C(8)	121·4 (0·3)	H(9)	C(8)	C(9)	114·0 (1·6)
C(5)	C(4)	C(9)	118·7 (0·3)	H(10)	C(9)	C(8)	121·9 (1·5)
C(5)	C(6)	C(7)	119·0 (0·3)	H(10)	C(9)	C(4)	116·7 (1·5)
C(6)	C(7)	C(8)	121·3 (0·3)				
C(7)	C(8)	C(9)	119·1 (0·3)				

Waber (1965) for K^+, S, C, O and N, and the hydrogen form factors were those of Stewart, Davidson & Simpson (1965).

Fig. 1. Bond distances and bond angles involving non-hydrogen atoms.

The final positional and thermal parameters and their estimated standard deviations are presented in Tables 2 and 3, and the observed and calculated structure factors in Table 4.
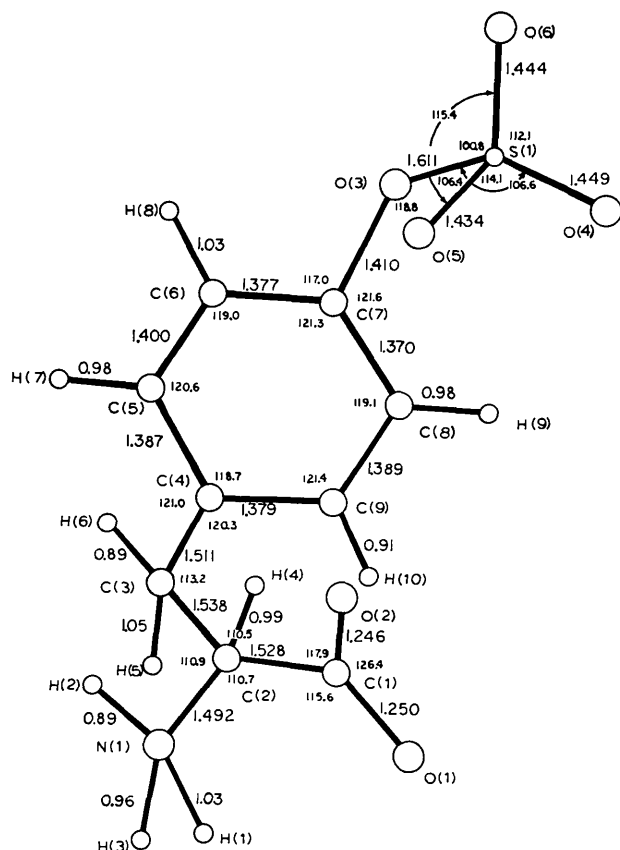
Description of the structure

Bond distances and angles

The bond lengths and bond angles together with their estimated standard deviations are listed in Tables 5 and 6 respectively, and shown in Fig. 1. The general appearance of the molecule and the relative thermal vibrations of its constituent atoms are illustrated in the *ORTEP* plot (Johnson, 1965) of Fig. 2.

It has been observed that, in amino acids, the carboxy carbon to C(2) bond [C(1)–C(2)] is significantly longer than a normal $C(sp^2)$–$C(sp^3)$ single bond (Putkey & Sundaralingam, 1970; Sundaralingam & Putkey, 1970). The mean value for amino acid C(1)–C(2) bonds in both the ionized and free acid forms is 1·530 Å. The corresponding bond length in the carboxylate ions is 1·490 Å and in the carboxylic acids and amides is 1·510 Å. In tyrosine sulfate the $C(1)sp^2$–$C(2)sp^3$ bond of 1·528 ± 0·004 Å, is significantly longer than the 1·50 Å normally associated with an sp^2–sp^3 bond. The $C(3)sp^3$–$C(4)sp^2$ bond distance is 1·511 ± 0·005 Å and is typical of similar bonds attached to the benzene ring.

The ring C–C bonds appear to be quite normal. Although the average C–C bond distance 1·384 Å, is close to the value found in crystalline benzene, the presence of the *para* substituents appears to produce the approximate bond length sequence: C(5)–C(6) = C(8)–C(9) (average 1·395 Å) > C(4)–C(5) = C(4)–C(9) (average 1·383 Å) > C(6)–C(7) = C(7)–C(8) (average 1·374 Å). Thus, the benzene ring possesses mirror symmetry across the C(4)–C(7) axis.

The carboxylate C $O^{\delta-}$ and the amino C–N^+ bond

Fig. 2. The ellipsoids of vibration for potassium L-tyrosine-O-sulfate dihydrate.

distances are within the normal range. The phenolic ester bond C(7)–O(3) is $1 \cdot 410 \pm 0 \cdot 004$ Å and compares well with the value of $1 \cdot 402$ Å found in acetylsalicylic acid (Wheatley, 1964). It is noteworthy that esterification produces a considerable increase in the phenolic C–O bond distance in comparison with the phenol C–O(H) bond distance of $1 \cdot 358 \pm 0 \cdot 004$ Å found in salicylic acid (Sundaralingam & Jensen, 1965a).
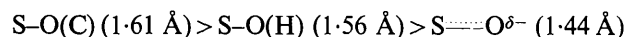
The bond distances and angles (Table 5) involving the hydrogen atoms are in the range expected for X-ray structures.

The bond angles in the ring at C(4), C(6) and C(8) are smaller than 120°, while those at C(5), C(7) and C(9) are greater than 120°. The angle at the amino acid end of the ring at C(4) is $2 \cdot 6°$ smaller than the angle at the sulfate carbon atom C(7). The exocyclic angle O(3)–C(7)–C(8) = 121·6° is 4·6° greater than the exocyclic angle O(3)–C(7)–C(6), because the molecule has lost some of the non-bonded interactions between the sulfate group and the aromatic ring atoms C(8), H(9) and C(7). However, the exocyclic angles at C(4) are approximately equal.

The C(4)–C(3)–C(2) valence angle, 113·2°, is significantly greater than the tetrahedral value. The valence angles at the asymmetric carbon atom and the carboxylate carbon atom are within the range of values generally observed for the amino acids (Marsh & Donohue, 1967; Sundaralingam & Putkey, 1970).

The sulfate group

The sulfate ester bond S–O(3) is $1 \cdot 611 \pm 0 \cdot 003$ Å, comparable with $1 \cdot 60$ Å in ethyl sulfate (Jarvis, 1953). The mean value of the remaining $S{=\!=}O^{\delta-}$ bonds is 1·442 Å, similar to 1·45 Å in ethyl sulfate. In the HSO_4^- ion (Taesler & Olovsson, 1968) the mean value, 1·448 Å, of the $S{=\!=}O^{\delta-}$ bonds is close to the above values, even though the HSO_4^- ion is not an ester and the S–OH bond distance is 1·56 Å. Thus, the S–O bond distances present the sequence

S–O(C) (1·61 Å) > S–O(H) (1·56 Å) > $S{=\!=}O^{\delta-}$ (1·44 Å)

A similar sequence is observed in the phosphate monoesters (Sundaralingam & Jensen, 1965b). In tyrosine sulfate the S–O(5) bond distance of 1·434 Å is significantly shorter than the S–O(4) and S–O(6) bonds. This may be due to the differences in the coordination distances to the K$^+$ ion. The distance O(5)–K$^+$ is 0·058 Å (18σ) longer than the O(6)–K$^+$ and O(7)–K$^+$ distances.

The O–S–O valence angles exhibit considerable departure from tetrahedral geometry. Such distortions are also observed in potassium ethyl sulfate. The largest O–S–O valence angle of 115·4° involves the anionic oxygen atoms; similar values are observed in ethyl sulfate and the HSO_4^- ion. The smallest valence angle of 100·8° involves the ester bond and the S–O(6) bond in tyrosine sulfate, while the smallest angle in HSO_4^- and ethyl sulfate involves the S–O(H,C) bond. The largest valence angle involves the $S{=\!=}O^{\delta-}$ bonds.

The valence angle at the ester oxygen atom,

S–O(3)–C(7), is 118·8°. Therefore, in many respects the geometry of the sulfate ester is similar to that of the phosphate ester (Cruickshank, 1961; Sundaralingam & Jensen, 1965b).

Planarity of the aromatic ring

The six carbon atoms constituting the benzene ring lie in a plane, Table 7. However the substituents C(3) and O(3) are significantly displaced on opposite sides from the plane.

Table 7. *The least-squares plane through the six atoms of the phenyl ring*

	Deviation	Substituent	Deviation
C(4)	$-0 \cdot 001$ Å	C(3)	$-0 \cdot 041$ Å
C(5)	0·005	C(3)	0·066
C(6)	$-0 \cdot 004$	H(7)	0·066
C(7)	$-0 \cdot 002$	H(8)	$-0 \cdot 083$
C(8)	0·006	H(9)	$-0 \cdot 067$
C(9)	$-0 \cdot 005$	H(10)	$-0 \cdot 043$
r.m.s. $\varDelta$	0·004		

Equation of plane:

$$-0 \cdot 6844X - 0 \cdot 6156Y - 0 \cdot 3907Z = 5 \cdot 5006$$

The equation of the plane is of the form $lX + mY + nZ = d$, where l, m and n are the direction cosines of the normal to the plane; and d (Å) is the distance of the plane from the origin, and X, Y, Z are coordinates in Å.

Conformation of the molecule

The torsional angles in the molecule are given in Table 8. Previous studies of tyrosine derivatives include glycyl-L-tyrosine hydrochloride (Smits & Wiebenga, 1953), L-tyrosine hydrochloride (Srinivasan, 1959) and diiodo-L-tyrosine dihydrate (Hamilton & Steinrauf, 1967). While they have not been particularly accurate, the conformation of N(1) and C(4) about the C(2)–C(3) bond, described by the torsional angle χ (Ramachandran, Edsall, Flory, Kendrew & Liguori, 1966), is generally *anti*, average $\bar{\chi} = 184 \cdot 9°$ (Table 9 and Fig. 3). However, the χ observed in this study is 71·8° corresponding to the *syn* conformation. Similar *syn* conformations have been reported in other metal complexes of tyrosine, copper glycyl-L-leucyl-L-tyrosinate (Van der Helm & Franks, 1968) and bis-L-tyrosinate copper(II) (Tatsch & Van der Helm, 1969). However, the functional groups responsible for coordination with copper are quite different from those coordinated to K$^+$. Thus, metal ions may influence the conformation of the tyrosine residue. It is noteworthy that phenylalanine hydrochloride (Gurskaya, 1964), a structural analog of tyrosine, also exhibits a +*syn* conformation $\chi_1 = 62 \cdot 2°$, similar to the metal complexes. These observations suggest that the conformational energies of the *syn* and *anti* forms are not markedly different, and that the *syn* $\rightleftarrows$ *anti* interconversion in tyrosine may be influenced by the molecular environment.

The conformation about the C(3)–C(4) bond χ_2, Table 9, shows a wide range of values, from 65 to 116°,

Table 8. *Torsion angles involving the nonhydrogen atoms of potassium L-tyrosine-O-sulfate dihydrate*

i	j	k	l	φ_{ijkl}
O(1)	C(1)	C(2)	N(1)	164·4°
O(1)	C(1)	C(2)	C(3)	−72·9
O(2)	C(1)	C(2)	N(1)	−18·2
O(2)	C(1)	C(2)	C(3)	104·5
N(1)	C(2)	C(3)	C(4)	71·9
C(1)	C(2)	C(3)	C(4)	−50·9
C(2)	C(3)	C(4)	C(5)	−82·7
C(2)	C(3)	C(4)	C(9)	95·5
C(3)	C(4)	C(5)	C(6)	177·8
C(3)	C(4)	C(9)	C(8)	−178·8
C(4)	C(5)	C(6)	C(7)	0·8
C(4)	C(9)	C(8)	C(7)	1·2
C(5)	C(6)	C(7)	C(8)	−0·6
C(5)	C(6)	C(7)	O(3)	176·4
C(5)	C(4)	C(9)	C(8)	−0·5
C(6)	C(7)	C(8)	C(9)	−0·9
C(6)	C(7)	O(3)	S(1)	127·6
C(7)	O(3)	S(1)	O(4)	81·0
C(7)	O(3)	S(1)	O(5)	−41·4
C(7)	O(3)	S(1)	O(6)	−162·1
C(8)	C(7)	O(3)	S(1)	−55·9
C(9)	C(8)	C(7)	O(3)	−177·3

(a)   (b)

(c)   (d)

Fig. 3. A comparison of the conformations of tyrosine derivatives as viewed down the C(2)-C(3) bond. (a) Potassium L-tyrosine sulfate dihydrate, (b) glycyl-L-tyrosine hydrochloride, (c) L-tyrosine hydrochloride, and (d) diiodo-L-tyrosine dihydrate.

O(2)-K⁺-O(4')	88.4°
O(2)-K⁺-O(5)	129.7°
O(4')-K⁺-O(5)	106 9°
O(4')-K⁺-O(6')	51 5°
O(5)-K⁺-O(6')	128 3°
O(6')-K⁺-O(7)	93.9°
O(7)-K⁺-O(2)	158.5°

Fig. 4. The pentacoordination of the K⁺ ion and the closest distances of the aromatic ring to it. The valence angles involving the potassium ion are also shown.

with an average value of 92°. Similar conformational preference about the exocyclic bond attached to the aromatic ring is exhibited by other molecules provided that the atom attached to the aromatic ring [C(3) in this case] is tetracovalent (Sundaralingam, 1969).

The amino nitrogen atom is 0·4 Å out of the plane of the carboxylate group, a common feature among the amino acids (Sundaralingam & Putkey, 1970). Furthermore, in the tyrosine derivatives the out-of-plane displacement of the nitrogen atom appears to be generally larger than in the other amino acids, as seen by the torsional angles O(2)-C(1)-C(2)-N(1), Table 9. This is probably correlated with non-bonded interaction between the carboxy oxygen atom and the aromatic ring. In the *anti* conformation about the C(2)-C(3) bond the torsional angle O(2)-C(1)-C(2)-N(1) is greater than 30°, while in the *syn* conformation it is less than 20°.

Table 9. *The torsional angles in tyrosine derivatives and analogs*

		1	2	3	4	5
χ_1	O(1)-C(1)-C(2)-N(1)	164·4	149·1	149·6	108·0	177·5
χ_2	O(2)-C(1)-C(2)-N(1)	341·8	326·6	323·9	301·6	358·1
θ	C(1)-C(2)-C(3)-C(4)	309·1	69·9	65·3	67·7	301·0
χ_1	N(1)-C(2)-C(3)-C(4)	71·8	189·8	185·2	179·6	62·2
χ_2	C(2)-C(3)-C(4)-C(5)	277·3	294·6	243·6	266·9	256·0

1 L-Tyrosine-O-sulfate.2H₂O (this investigation).
2 Glycyl-L-tyrosine.HCl (Smits & Wiebenga, 1953)
3 L-Tyrosine.HCl (Srinivasan, 1959).
4 Diiodo-L-tyrosine.2H₂O (Hamilton & Steinrauf, 1967).
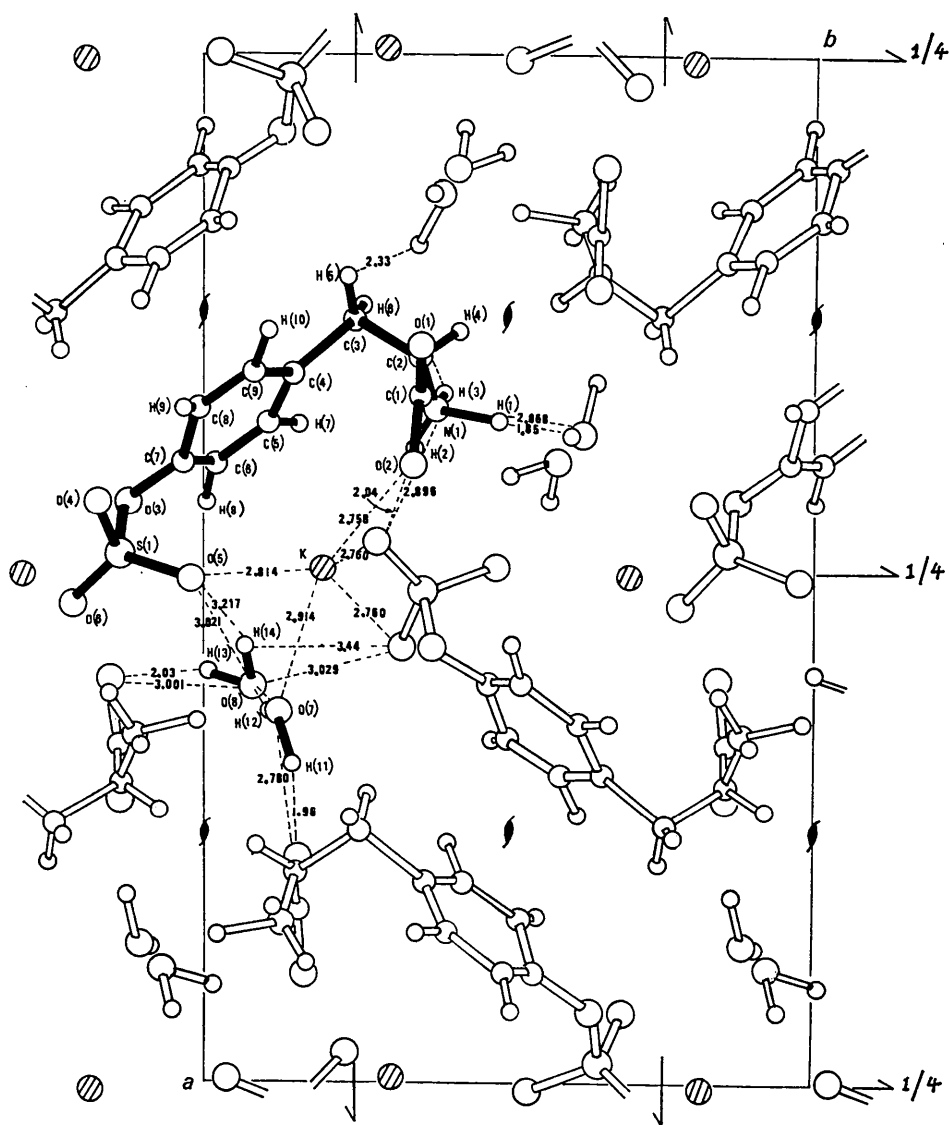5 L-Phenylalanine.HCl (Gurskaya, 1964).

Fig. 5. A packing diagram projected down the c axis. H(14) is the 'free' proton.

Table 10. *The hydrogen bond distances and angles*

Symmetry code

	x	y	z
a	x	y	z
b	x	y	$z-1$
c	$\frac{1}{2}+x$	$\frac{1}{2}-y$	$1-z$
d	$1-x$	$\frac{1}{2}+y$	$\frac{1}{2}-z$
e	$1-x$	$y-\frac{1}{2}$	$\frac{1}{2}-z$

Reference molecule						Distances			Angles		
A_1	A_2	H	Code	A_3	A_4	A_2–H	A_3–H	A_2–A_3	$A_1A_2A_3$	A_2HA_3	$A_2A_3A_4$
H(11)	O(7)	H(12)	b	O(8)	H(13)	0·77 Å	2·17 Å	2·788 Å	113·9°	149·5°	83·0°
H(12)	O(7)	H(11)	c	O(1)	C(1)	0·86	1·96	2·780	80·4	159·6	129·5
H(14)	O(8)	H(13)	e	O(2)	C(1)	0·76	2·03	3·001	71·8	136·3	87·5
C(2)	N(1)	H(1)	d	O(7)	H(11)	1·03	1·85	2·868	102·5	164·3	94·9
C(2)	N(1)	H(2)	d	O(6)	S(1)	0·84	2·04	2·896	126·1	169·8	107·9
C(2)	N(1)	H(3)	b	O(1)	C(1)	0·99	1·83	2·785	108·2	167·4	115·3

Crystal packing

In general, the molecular packing within the lattice is dominated by ionic interactions among the potassium ions and the ionic sites of the amino acid.

Potassium coordination

Tyrosine sulfate binds to potassium ions as a bidentate chelate, the potassium ion being coordinated to both a sulfate oxygen atom, O(5), and the carboxyl oxygen atom, O(2). The penta-coordination of potassium heretofore unobserved in crystal structure analyses is completed by the two remaining sulfate oxygen atoms, O(4) and O(6), in symmetry-related positions and a water molecule, O(7) (Fig. 4). It may be significant that the mean O–K$^+$ distance of 2·801 Å is intermediate between the mean values of 2·787 Å for four-coordinate and 2·830 Å for six-coordinate potassium ions (*International Tables for X-ray Crystallography*, 1962). Fig. 4 also illustrates the disposition of the potassium ion relative to the aromatic ring. The minimum contact distance of 3·532 Å indicates little or no interaction between the ion and the ring.

Hydrogen bonding

In addition to the K$^+\cdots$O$^{\delta-}$ coordinate bonds the crystal structure possesses six hydrogen bonds, three O–H$\cdots$O and three N–H$\cdots$O, see Table 10 and Figs. 5 and 6. The O–H$\cdots$O hydrogen bonds involve the two O–H groups of the hydrate oxygen atom O(7) and one O–H group of the hydrate oxygen atom O(8). The acceptor oxygen atoms are the carboxylate O(1), O(2), and the hydrate O(8). The latter is a water to water
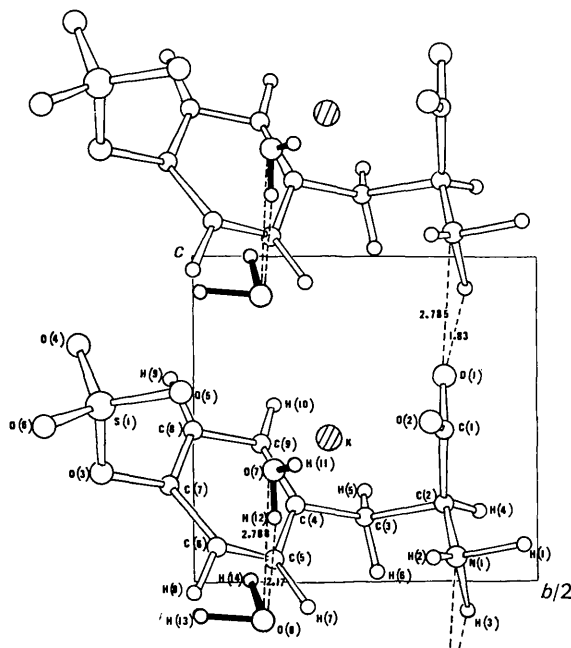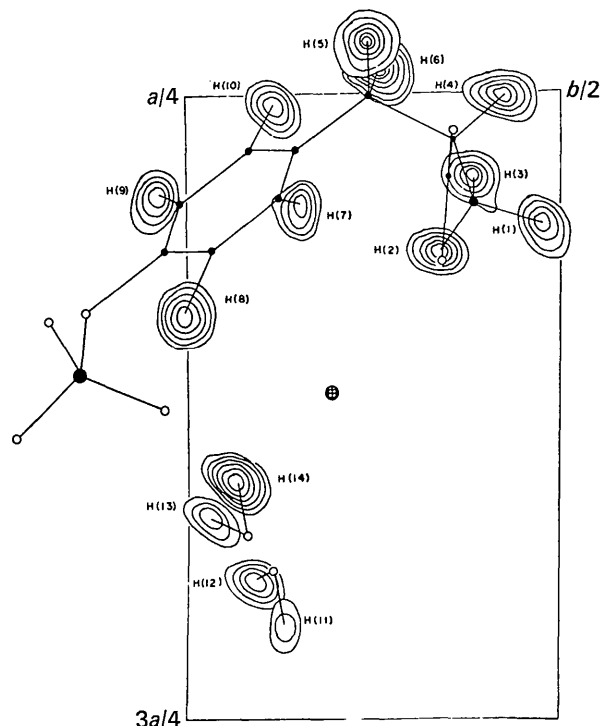

Fig. 7. Difference electron density map showing all the hydrogen atoms, including the hydrogen atom H(14) which is not involved in any hydrogen bonds.

hydrogen bond. Hence, O(7) is involved in two donor hydrogen bonds, one acceptor N–H$\cdots$O(7) hydrogen bond and a coordinate bond to K$^+$. O(8) is involved only in a donor and an acceptor hydrogen bond.

H(14), not involved in hydrogen bonding, appeared in the difference electron density map, Fig. 7, at a peak density of 0·22 e.Å^{-3}, attached to an oxygen atom [O(8)] with an isotropic B value of 7·2 Å^2, while a difference electron density synthesis subtracting all the atoms revealed no residual densities greater than 0·08 e.Å^{-3}. Furthermore, the characteristic peak in the Raman spectrum of the solid at 3602 cm^{-1} corroborates the existence of a free (not involved in hydrogen bonding) O–H group (Hamilton & Ibers, 1968). Although O(8) is 3·021 Å from O(5) and 3·029 Å from O(4), its hydrogen atom H(14) is over 3 Å from either of these atoms.

The three N–H$\cdots$O hydrogen bonds are between the ammonium group and the oxygen atoms O(1) of the carboxylate, O(6) of the sulfate and O(7) of the hydrate. The N–H$\cdots$O hydrogen bond angles are closer to being linear than the O–H$\cdots$O angles, Table 10.

As is generally observed in esters, the sulfate ester oxygen atom, O(3), is not in any hydrogen bond nor does it exhibit any short intermolecular contact (Sundaralingam, 1969). Among the anionic oxygen atoms, only O(6) is involved in a hydrogen bond while the rest are coordinated to potassium ions.

Fig. 6. The structure viewed down the *a* axis.

Conclusion

The biological significance of tyrosine sulfate is not known. Human fibrinopeptide contains the acidic phosphate ester function as the O-phosphoserine while most other species contain the acidic sulfate ester (Blomback, Blomback & Edman, 1962). The similarity in the bond distances, bond angles, conformation and metal binding (Na^+, K^+, Ca^{2+}) of phosphate and sulfate esters may have some significance in their biological function in the fibrinopeptides. However, the monoanionic sulfate can only accept hydrogen bonds, whereas the monoanionic phosphate can donate one hydrogen bond, in addition to accepting several hydrogen bonds.

Mutt & Jorpes (1958) found the biological activity of cholecystokininpancreozymin to be localized within the C-terminal octapeptide obtained by trypsin digestion of the hormone. Both the hormones gastrin and cholecystokininpancreozymin contain the sulfated tyrosine within the C-terminal heptapeptide, while the B fibrinopeptides contain them within the N-terminal heptapeptide.

Perhaps the hormones acquire their biologically active conformation by virtue of an intramolecular hydrogen bond between the sulfate and peptide NH group of the backbone or the functional groups of the chains producing a compact structure for the peptide chain. In addition, the sulfate can form hydrogen bonds and coordinate bonds to membrane proteins and metal ions, and thus be associated with membrane transport which may be linked to the dual conformational property of the tyrosine residue discussed above.

In fibrinopeptide B, in addition to possible intrachain hydrogen bonds, interchain hydrogen bonds between B chain-B chain and B-chain-A chain of fibrinogen may occur to give the complex a fairly compact structure.

The following computer programs were used in this investigation.

Busing, Martin & Levy (1962) least-squares program modified for the Univac 1108 computer (Rao, 1968). ORTEP thermal ellipsoid plot program (Johnson, 1965). The other programs were written in this laboratory (Rao, 1968).

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