Conformations of Penicillin G: Crystal Structure of Procaine Penicillin G Monohydrate and a Refinement of the Structure of Potassium PenicillinG

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The geometric details and mode of puckering of the five-membered (thiazolidine) ring have been found to be different in two penicillin G salts. In procaine penicillin G monohydrate (aqueous procaine penicillin G or APPG) the penicillin molecule is in an ' extended ' form whereas it is ' coiled ' in potassium penicillin G. The solid-state conformation of penicillin G therefore depends more on local environment than on nature of substitution in the side chain. The procaine molecules in APPG adopt an enlarged (91°) torsion angle in the O–C–C–N group and exhibit little quinonoid character in the *p*-aminobenzoate group.

PENICILLINS are of immense medical and commercial value. Through the acylating ability of the labile β-lactam, adequate concentrations of these molecules will kill a wide variety of bacteria. However, treatment reactions can extend from allergic responses which are typical of penicillins in general, to a dramatic, but non-fatal ' toxic ' or ' psychotic ' reaction peculiar to aqueous procaine penicillin G.[1-4]

Aqueous procaine penicillin G (APPG) is a depot preparation which, by means of relative insolubility in body tissues, provides persistent and stable blood levels of penicillin G. It is the agent of choice for treating gonococcal infections because, among other reasons, the same course of therapy which eliminates the gonococci can abort other, simultaneously acquired infections. Current treatment schedules for gonorrhea require substantial doses (4.8 million units, *ca.* 8 cc in aqueous suspension) of APPG which can lead to an unacceptable frequency of toxic treatment reactions with the result that less efficacious or more expensive medications may be substituted.

We undertook the structure determination of aqueous procaine penicillin G to study the effects of association on the conformations of both the penicillin G and procaine molecules and hopefully to gain insight into the unusual biological effects of this compound.

To make the contrasts and comparisons more relevant, we re-examined potassium penicillin G (KPG) using a new set of diffractometer data.[5,6]

EXPERIMENTAL

Aqueous Procaine Penicillin G.—Crystal data for APPG are given in Table 1. Crystals, readily grown by evaporation from ethanol, are colourless and bounded principally by (100), ($\bar{1}$00), (001), 00$\bar{1}$), (0$\bar{1}$0), 011), and (01$\bar{1}$). A specimen (obtained from commercial therapeutic material) *ca.* 0.28 × 0.22 × 0.17 mm was mounted with *b* near the ϕ axis of a Picker FACS 1 computer-controlled diffractometer. The intensities of the 2 812 unique reflections accessible to nickel-filtered Cu-K_α radiation ($2\theta_{max.}$ 130°) were measured with θ—2θ scans at 2° min^{-1}. Backgrounds at both ends of the scan were counted for 10 s. The 2 718 reflections having $I_o > 2\sigma(I_o)$ (based on counting statistics) were considered observed. Corrections for absorption were made and A^* ranged to 1.27 from 1.20.

The structure was determined from a series of E maps using phases refined by the tangent formula after the sulphur atom was located from an $E^2 - 1$ vector map. Least-squares refinement proceeded with isotropic and then anisotropic temperature factors to R 0.11. Hydrogen atoms were then located in a difference map and included in the refinement with isotropic temperature factors.

A weighting scheme of the form $w = 1.0$ for $F_o < 8.0$, and $w = 8.0/F_o$ for $F_o \geqslant 8.0$ was introduced as were anomalous dispersion corrections for sulphur and refinement completed with 507 variables divided into 27 blocks. The final R and weighted factors R' were both 0.073. The final difference map showed no peak higher than 0.3 eÅ^{-3}. The *X*-Ray '72 System modified for use on a PDP 10 computer was used for most calculations.[7]

Potassium Penicillin G.—Crystal data for KPG are in Table 1. Yellow orthorhombic plates showing (010), (0$\bar{1}$0), ($\bar{1}$00), (100), (001), and (00$\bar{1}$) development were grown by evaporation from methanol. A specimen, 0.325 × 0.150 × 0.50 mm, was mounted in arbitrary orientation on a Syntex $P2_1$ diffractometer. By use of monochromatized Mo-K_α radiation 1 875 reflections were measured using 1° min^{-1} θ–2θ scans with the measurement time of each reflection equally divided between peak and background. The intensities of 1 272 reflections had $I > 1.50\sigma(I_o)$. A^* ranged from 1.06 to 1.02 in the absorption correction.

The KPG model of Pitt [5] was quickly confirmed and least-squares refinement with isotopic and then anisoptic temperature factors followed. Hydrogen atoms were included (with fixed isotropic thermal factors) at calculated positions and the weighting scheme $w = 0.15\ F_o$ was introduced. After the introduction of anomalous dispersion corrections for sulphur and potassium, the function minimized being $\Sigma w(|F_o| - |F_c|)^2$, converged with R 0.072 and R' 0.084. The difference map showed several peaks >0.3 eÅ^{-3}, but these were associated with two heaviest atoms. ' Ghost ' peaks related to the positions of the potassium and sulphur atoms, as described by Pitt,[5] did not appear.

RESULTS

Co-ordinates obtained in these refinements are presented in Table 2, bond distances and angles in Table 3. The atom numbering system (Figures 1 and 2) is that used by

[1] R. C. Batchelor, G. O. Horne, and H. L. Rogerson, *Lancet*, 1951, 195.
[2] P. M. Utley, J. B. Lucas, and T. E. Billings, *Southern Med. J.*, 1966, 1271.
[3] R. Tompsett, *Arch. Intern. Med*, 1967, 565.
[4] Y. Moene, G. Aimard, M. T. Perrenin, and G. Ramband, *J. Med. Lyon*, 1971, 1323.
[5] G. J. Pitt, *Acta Cryst.*, 1972, 770.
[6] A. Vaciago, *Atti. Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat.*, 1960, 851.
[7] ' *X*-Ray ' Program System, Technical Report TR 192, Computer Science Center, University of Maryland, June 1972.

TABLE 1

Crystal data for aqueous procaine penicillin G (APPG) and potassium penicillin G (KPG)

	APPG	KPG
Formula	$C_{29}H_{40}N_4O_7S$	$C_{16}H_{17}KN_2O_4S$
M	588.71	372.47
Space group	$P2_1$	$P2_12_12_1$
U/Å$_3$	1 552.4	1 770.9
D_m/g cm^{-3}	1.259	1.359
Z	2	4
μ/cm^{-1}	10.51 (Cu-K_α)	3.45 (Mo-K_α)
a/Å	10.672(2)	9.303(2)
b/Å	10.446(1)	6.342(3)
c/Å	15.595(3)	30.015(9)
β/°	116.74(2)	

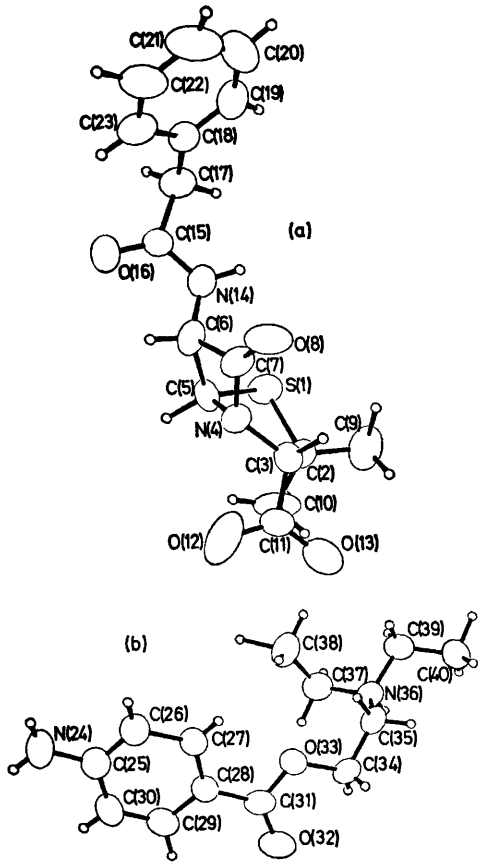

FIGURE 1 Atomic labelling of the species in aqueous procaine penicillin G (APPG) (a) penicillin G (b) procaine

Sweet.[8] Observed and calculated structure factors and thermal parameters are listed in Supplementary Publication No. SUP 22120 (39 pp.).*

Aqueous Procaine Penicillin G.—Bond lengths and angles for the species in APPG are essentially similar to those reported in other investigations.

(*a*) *Penicillin G.* The five-membered-ring in the penicillin molecule exhibits several interesting features. First, the interior angle at the sulphur atom is 91.8(2)°, intermediate between the 89.8° of ampicillin and the 96° of 6-aminopenicillanic acid (6-APA) and penicillin V.[8] Sweet

* See Notice to Authors No. 7 in *J.C.S. Perkin I*, 1977, Index issue.

[8] R. M. Sweet, in ' Cephalosporins and Penicillins ', Academic Press, New York, 1972, pp. 280—309.

TABLE 2

Co-ordinates of aqueous penicillin G, with estimated standard deviations obtained from the least-squares refinements in parentheses

(*a*) Penicillin G

Atom	X	Y	Z
S(1)	0.424 9(1)	0.300 7	0.052 6(1)
C(2)	0.546 3(5)	0.177 0(5)	0.127 9(3)
C(3)	0.481 6(5)	0.045 4(5)	0.076 5(3)
N(4)	0.335 1(4)	0.070 1(4)	0.011 1(3)
C(5)	0.277 3(5)	0.194 7(6)	0.017 0(3)
C(6)	0.180 6(5)	0.183 5(6)	0.906 6(4)
C(7)	0.277 9(5)	0.074 1(5)	0.911 5(3)
O(8)	0.305 0(5)	0.018 9(4)	0.854 4(3)
C(9)	0.689 2(7)	0.199 6(6)	0.132 3(6)
C(10)	0.553 4(8)	0.182 6(7)	0.228 4(4)
C(11)	0.501 3(5)	0.437 3(5)	0.853 1(3)
O(12)	0.604 5(4)	0.424 3(5)	0.844 0(3)
O(13)	0.381 9(4)	0.391 2(4)	0.807 7(3)
N(14)	0.164 7(4)	0.288 9(5)	0.843 0(3)
C(15)	0.038 1(5)	0.337 0(5)	0.787 2(3)
O(16)	0.932 9(4)	0.308 9(6)	0.793 9(3)
C(17)	0.034 7(6)	0.430 7(6)	0.711 5(4)
C(18)	−0.007 0(5)	0.367 1(5)	0.615 7(3)
C(19)	0.063 7(6)	0.396 3(8)	0.563 5(5)
C(20)	0.023 1(9)	0.341(1)	0.473 4(6)
C(21)	0.912 9(9)	0.255(1)	0.438 1(5)
C(22)	0.843 2(8)	0.226 6(8)	0.487 5(5)
C(23)	0.879 0(7)	0.281 3(8)	0.576 4(4)
H(3)	0.534(5)	0.024(5)	0.035(3)
H(5)	0.219(5)	0.198(5)	0.057(4)
H(6)	0.092(5)	0.160(6)	0.898(3)
H(10A)	0.449(7)	0.183(9)	0.205(5)
H(10B)	0.627(8)	0.103(8)	0.261(6)
H(10C)	0.586(8)	0.262(8)	0.244(6)
H(9A)	0.76(1)	0.12(1)	0.153(7)
H(9B)	0.66(1)	0.18(1)	0.040(7)
H(9C)	0.73(1)	0.26(1)	0.161(7)
H(14)	0.236(6)	0.309(7)	0.832(4)
H(17A)	−0.033(7)	0.490(8)	0.705(5)
H(17B)	0.128(7)	0.466(7)	0.739(5)
H(23)	0.928(6)	0.320(7)	0.600(4)
H(22)	0.739(8)	0.196(9)	0.450(5)
H(21)	0.905(9)	0.211(9)	0.387(6)
H(20)	0.068(8)	0.365(9)	0.445(5)
H(19)	0.144(4)	0.455(5)	0.599(3)

(*b*) Procaine and water

N(24)	0.009 4(5)	0.188 7(7)	0.095 0(3)
C(25)	0.098 0(5)	0.240 7(6)	0.183 3(4)
C(26)	0.207 6(6)	0.165 0(6)	0.251 2(4)
C(27)	0.294 9(6)	0.214 4(5)	0.341 1(3)
C(28)	0.278 0(5)	0.339 4(5)	0.363 5(3)
C(29)	0.172 0(6)	0.413 8(6)	0.296 7(4)
C(30)	0.084 1(6)	0.363 9(7)	0.207 5(4)
C(31)	0.367 8(6)	0.393 9(5)	0.459 7(4)
C(32)	0.625 0(5)	0.005 4(4)	0.519 4(3)
C(33)	0.444 0(4)	0.302 9(4)	0.522 5(2)
C(34)	0.538 2(7)	0.345 4(6)	0.618 1(4)
C(35)	0.595 6(6)	0.227 0(6)	0.677 2(3)
C(36)	0.511 8(5)	0.181 6(5)	0.726 6(3)
C(37)	0.359 8(7)	0.156 4(7)	0.661 5(4)
C(38)	0.335 1(8)	0.046 9(8)	0.593 6(5)
C(39)	0.579 5(7)	0.068 8(6)	0.7901 (4)
C(40)	0.718 9(8)	0.099 0(7)	0.874 9(5)
H(24A)	0.031(7)	0.122(7)	0.080(5)
H(24B)	−0.050(7)	0.234(8)	0.052(5)
H(26)	0.231(7)	0.094(7)	0.233(5)
H(27)	0.366(6)	0.163(6)	0.385(4)
H(29)	0.164(5)	0.499(5)	0.315(3)
H(30)	0.015(7)	0.402(7)	0.163(4)
H(34A)	0.619(6)	0.383(6)	0.623(4)
H(34B)	0.486(7)	0.417(8)	0.644(5)
H(35A)	0.594(7)	0.165(8)	0.636(5)
H(35B)	0.694(5)	0.245(5)	0.725(3)
H(37A)	0.307(7)	0.138(8)	0.707(5)
H(37B)	0.322(6)	0.244(6)	0.626(4)
H(38A)	0.377(6)	0.070(6)	0.555(4)

TABLE 2 (*Continued*)

Atom	*X*	*Y*	*Z*
H(36)	0.514(7)	0.250(7)	0.766(5)
H(38B)	0.37(1)	−0.04(1)	0.632(7)
H(38C)	0.20(1)	0.01(1)	0.542(7)
H(39A)	0.514(6)	0.037(6)	0.806(4)
H(39B)	0.610(8)	0.003(9)	0.766(6)
H(40A)	0.799(8)	0.108(9)	0.865(6)
H(40B)	0.684(8)	0.166(9)	0.907(6)
H(40C)	0.752(9)	0.02(1)	0.924(7)
O(41)	0.896 1(4)	0.420 8(5)	0.948 8(3)
H(41A)	0.799(5)	0.427(5)	0.925(4)
H(41B)	0.90(1)	0.38(1)	0.913(7)
(*c*) Potassium penicillin G			
S(1)	0.125 9(5)	0.375 0(3)	0.385 6(1)
C(2)	−0.160(2)	0.361(1)	0.375 8(3)
C(3)	−0.188(1)	0.337 4(9)	0.324 6(3)
N(4)	−0.005(1)	0.405 5(8)	0.303 9(2)
C(5)	0.194(1)	0.406(1)	0.327 5(3)
C(6)	0.229(2)	0.563(1)	0.310 7(3)
C(7)	0.008(2)	0.549(1)	0.292 0(3)
O(8)	−0.124(1)	0.627 2(8)	0.275 7(3)
C(10)	−0.253(2)	0.238(1)	0.403 2(3)
C(9)	−0.265(2)	0.504(1)	0.389 1(4)
C(11)	−0.206(2)	0.176 8(9)	0.310 7(3)
O(12)	−0.040(1)	0.107 0(7)	0.308 8(2)
O(13)	−0.388(1)	0.129 9(7)	0.303 7(2)
N(14)	0.256(1)	0.672 7(7)	0.343 3(2)
C(15)	0.323(2)	0.805(1)	0.332 2(4)
O(16)	0.373(2)	0.832 4(8)	0.293 8(2)
C(17)	0.333(2)	0.915(1)	0.368 8(4)
C(18)	0.282(2)	0.870(1)	0.413 7(3)
C(19)	0.428(3)	0.811(2)	0.441 0(4)
C(2)	0.375(4)	0.751(3)	0.482 8(6)
C(21)	0.181(4)	0.773(3)	0.496 5(6)
C(22)	0.034(3)	0.821(3)	0.472 5(6)
C(23)	0.090(3)	0.872(2)	0.431 2(5)
K+(24)	0.274 7(3)	0.099 3(2)	0.248 7(1)
H(3)	−0.34(1)	0.396(9)	0.310(3)
H(5)	0.30(1)	0.354(9)	0.321(3)
H(6)	0.34(1)	0.58(1)	0.290(3)
H(9A)	−0.15(2)	0.157(9)	0.398(3)
H(9B)	−0.37(2)	0.22(1)	0.399(3)
H(9C)	−0.20(2)	0.25(1)	0.435(3)
H(10A)	−0.43(2)	0.49(1)	0.388(3)
H(10B)	−0.25(2)	0.568(9)	0.371(3)
H(10C)	−0.24(2)	0.52(1)	0.419(3)
H(14A)	0.19(1)	0.681(9)	0.368(3)
H(17A)	0.22(2)	0.99(1)	0.361(3)
H(17B)	0.51(1)	0.96(1)	0.364(3)
H(19)	0.58(1)	0.801(9)	0.430(3)
H(20)	0.51(1)	0.69(1)	0.493(3)
H(21)	0.18(1)	0.74(1)	0.527(3)
H(22)	−0.13(2)	0.836(9)	0.485(3)
H(23)	0.01(1)	0.93(1)	0.419(3)

has discussed the fact that four of the five atoms in the thiazolidine ring tend to be co-planar. In APPG the best plane (in the χ^2 sense) * is formed by atoms C(2),C(3),N(4),-C(5) [χ^2 199 and σ 0.04 Å; for the next best plane, S(1),C(2),C(3),N(4), χ^2 is 1 190 and σ 0.11 Å]. The sulphur atom is displaced 0.7 Å on the same side of this plane as the β-lactam group. Methyl group C(10) [on C(2)] is on the same side of the C(2),C(3),N(4),C(5) plane as the carboxy-carbon [C(11)]. A torsion angle, C(10)–C(2)–C(3)–C(11), of only 24° combined with a separation of 2.893(8) Å show that these two atoms are in a nearly eclipsed conformation.

The geometry of the β-lactam in APPG is typical of this group in antibiotics. N(4) is 0.40 Å from the plane of its substituents [C(3),C(5),C(7)] and the sum of bond angles about N(4) is 336.7(7)°. Presumably, this lack of planarity

* $\chi^2 = \sum_{i=1}^{n} [P_i^2/\sigma^2(P_i)]$ where N = number of atoms in the plane, $\sigma^2(P_i) = l^2\sigma^2(X_i) + m^2\sigma^2(Y_i) + n^2\sigma^2(Z_i)$, and $P_i = lX_i + mY_i + nZ_i - P$ = distance of point (X_i, Y_i, Z_i) from the plane.

TABLE 3

Bond distances and angles

	APPG	KPG
(*a*) Penicillin G		
(*i*) Distances (Å)		
S(1)–C(2)	1.833(5)	1.847(10)
S(1)–C(5)	1.798(6)	1.818(9)
C(2)–C(3)	1.584(7)	1.57(1)
C(3)–N(4)	1.455(5)	1.46(1)
N(4)–C(5)	1.460(8)	1.45(1)
N(4)–C(7)	1.389(6)	1.38(1)
C(5)–C(6)	1.563(7)	1.56(1)
C(6)–C(7)	1.523(8)	1.52(2)
C(7)–O(8)	1.200(8)	1.21(1)
C(2)–C(9)	1.535(9)	1.52(2)
C(2)–C(10)	1.514(10)	1.53(2)
C(3)–C(11)	1.528(7)	1.55(1)
C(11)–O(12)	1.222(8)	1.23(1)
C(11)–O(13)	1.243(6)	1.25(1)
C(6)–N(14)	1.438(8)	1.43(1)
N(14)–C(15)	1.333(6)	1.34(1)
C(15)–O(16)	1.209(8)	1.22(1)
C(15)–C(17)	1.519(8)	1.51(2)
C(17)–C(18)	1.509(8)	1.45(2)
C(18)–C(23)	1.410(9)	1.33(2)
C(23)–C(22)	1.385(10)	1.37(3)
C(22)–C(21)	1.324(15)	1.28(3)
C(21)–C(20)	1.377(13)	1.32(3)
C(20)–C(19)	1.398(12)	1.41(3)
(ii) Angles (°)		
C(2)–S(1)–C(5)	91.8(2)	95.2(4)
S(1)–C(2)–C(3)	105.4(3)	106.2(6)
C(9)–C(2)–C(10)	111.2(5)	110(1)
C(2)–C(3)–N(4)	106.7(4)	105.2(7)
C(2)–C(3)–C(11)	113.1(4)	113.9(7)
N(4)–C(3)–C(11)	112.2(4)	111.3(7)
C(3)–N(4)–C(5)	117.7(4)	119.4(7)
C(3)–N(4)–C(7)	125.6(5)	125(1)
C(5)–N(4)–C(7)	93.4(4)	94.1(8)
S(1)–C(5)–N(4)	103.4(3)	105.1(6)
S(1)–C(5)–C(6)	116.0(4)	119.7(6)
N(4)–C(5)–C(6)	87.3(4)	88.3(7)
C(5)–C(6)–C(7)	84.4(4)	84.1(7)
C(5)–C(6)–N(14)	121.2(5)	117.3(7)
C(7)–C(6)–N(14)	118.4(5)	115(1)
N(4)–C(7)–C(6)	91.5(4)	93(1)
N(4)–C(7)–O(8)	133.1(5)	131(1)
C(6)–C(7)–O(8)	135.2(5)	137(1)
C(3)–C(11)–O(12)	118.0(4)	117(1)
C(3)–C(11)–O(13)	117.1(5)	116(1)
O(12)–C(11)–O(13)	124.8(5)	127(1)
C(6)–N(14)–C(15)	120.6(5)	121.1(1)
N(14)–C(15)–C(17)	114.6(5)	117(1)
C(17)–C(15)–O(16)	121.5(5)	122(1)
N(14)–C(15)–O(16)	124.0(5)	121(1)
C(15)–C(17)–C(18)	112.3(5)	118(1)
C(17)–C(18)–C(23)	121.3(6)	124(1)
C(17)–C(18)–C(19)	119.8(5)	122(1)
C(18)–C(23)–C(22)	119.5(7)	128(2)
C(23)–C(22)–C(21)	121.0(8)	115(2)
C(20)–C(21)–C(22)	121.2(8)	125(2)
C(21)–C(20)–C(19)	119.6(9)	118(2)
C(20)–C(19)–C(18)	120.0(7)	120(2)
C(23)–C(18)–C(19)	118.8(6)	114(1)
(b) Procaine		
(i) Distances (Å)		
N(24)–C(25)	1.383(7)	1.359(4)
C(25)–C(26)	1.414(7)	1.405(4)
C(25)–C(30)	1.368(10)	1.407(5)
C(26)–C(27)	1.387(7)	1.367(4)
C(27)–C(23)	1.384(8)	1.396(4)
C(28)–C(29)	1.381(7)	1.409(4)
C(28)–C(31)	1.483(7)	1.457(4)
C(29)–C(30)	1.383(8)	1.356(5)
C(31)–O(32)	1.209(7)	1.210(4)
C(31)–O(33)	1.345(6)	1.356(4)
O(33)–C(34)	1.442(6)	1.427(6)

TABLE 3 (*Continued*)

	APPG	Procaine HCL*
C(34)–C(35)	1.499(8)	1.503(5)
C(35)–N(36)	1.496(9)	1.505(4)
N(36)–C(37)	1.500(7)	1.506(4)
N(36)–C(39)	1.499(7)	1.512(4)
C(37)–C(38)	1.499(11)	1.509(5)
C(39)–C(40)	1.514(8)	1.511(6)
(ii) Angles (°)		
N(24)–C(25)–C(26)	119.4(6)	121.2(3)
N(24)–C(25)–C(30)	122.2(5)	121.8(5)
C(26)–C(25)–C(30)	118.4(5)	116.9(3)
C(25)–C(26)–C(27)	120.2(6)	121.1(3)
C(26)–C(27)–C(28)	120.1(5)	121.9(3)
C(27)–C(28)–C(29)	119.8(4)	116.8(4)
C(27)–C(28)–C(31)	121.2(4)	123.7(3)
C(29)–C(28)–C(31)	119.0(5)	119.5(3)
C(28)–C(29)–C(30)	120.1(6)	121.7(3)
C(29)–C(30)–C(25)	121.5(5)	121.5(3)
C(28)–C(31)–O(32)	127.6(5)	126.2(3)
C(28)–C(31)–O(33)	111.7(4)	112.9(3)
O(32)–C(31)–O(33)	119.4(4)	121.0(3)
C(31)–O(33)–C(34)	116.4(4)	116.8(2)
O(33)–C(34)–C(35)	106.5(4)	104.7(3)
C(34)–C(35)–N(36)	113.9(5)	116.1(3)
C(35)–N(36)–C(37)	114.9(4)	114.5(2)
C(35)–N(36)–C(39)	111.6(5)	112.1(2)
C(37)–N(36)–C(39)	111.3(5)	112.7(2)
N(36)–C(37)–C(38)	113.9(6)	112.2(3)
N(36)–C(39)–C(40)	113.9(5)	112.2(3)

* From ref. 9.

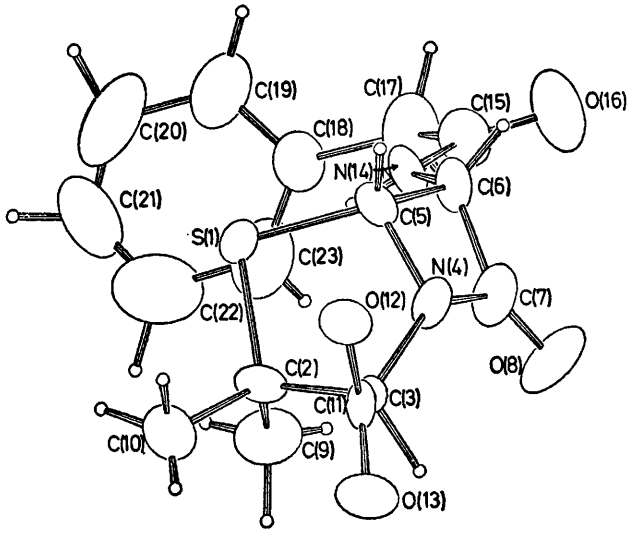

FIGURE 2 Atomic labelling of penicillin G in potassium penicillin G (KPG)

reduces electron delocalization into the N(4)–C(7) bond and weakens it. The N(4)–C(7) distance is 1.389(6) and C(7)–O(8) 1.200(8) Å, both values consistent with the lability of this group.[8] Atoms N(4),C(6),C(7),O(8) are more nearly co-planar (χ^2 38) than those forming the four-membered ring (χ^2 1 095) with C(5) displaced 0.41 Å on the same side of the better plane as the sulphur atom.

(*b*) *Procaine.* In the procaine molecule effects of association are extensive. The most obvious is an enlarged torsion angle O(33)–C(34)–C(35)–N(36) (Figure 3). While angles ranging from 60 to 70° have been found in other procaine salts,[9] in APPG this angle is 91.2(6)°.

[9] D. D. Dexter, *Acta Cryst.*, *B*, 1972, 77.

The other major effect is a diminution of quinonoid character in the *p*-aminobenzoate group compared to that in procaine hydrochloride. In the latter the quinonoid alternation of single and double bonds extends from the *p*-amino-group through the phenyl ring to the ester link in the side chain [see Table 3(*b*)] In APPG the N(24)–C(25),

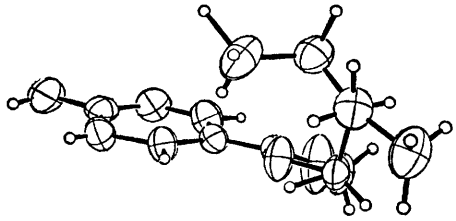

FIGURE 3 Procaine in APPG showing the nearly eclipsed hydrogen atoms which result from a torsional angle of 91° about the C(34)–C(35) bond

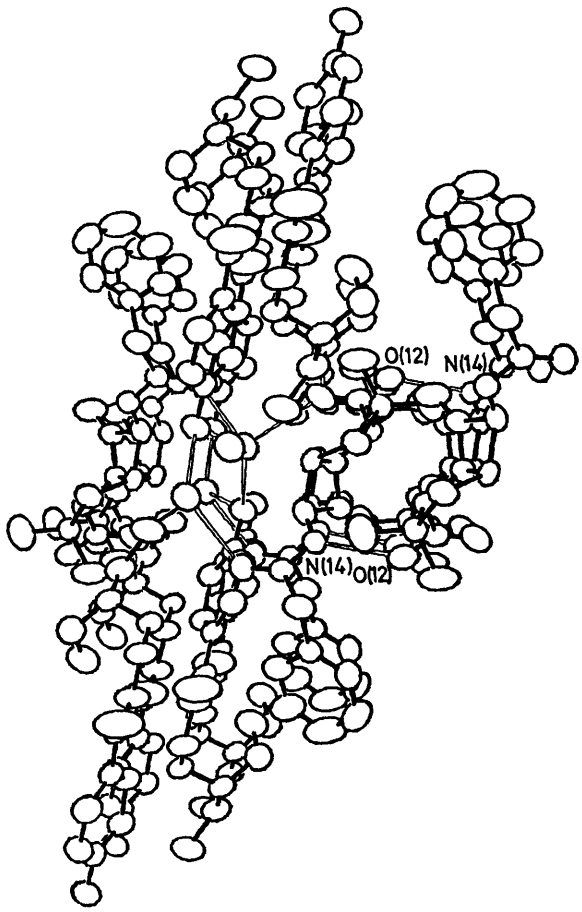

FIGURE 4 Molecular packing in APPG. Viewed down the *b* axis, the formation of a penicillin hydrogen-bond helical chain is apparent

C(28)–C(31), and O(33)–C(34) bonds are all longer than the corresponding bonds in procaine hydrochloride; the oxycarbonyl group of the ester is rotated 19° (compared with 7.4° in the hydrochloride) out of the plane of the phenyl ring; bond distances and angles within the phenyl group more nearly approximate to those of a regular hexagon.

(*c*) *Hydrogen bonding.* As is normal in crystals of penicillin compounds, the hydrogen bonding in APPG is

complex (Figure 4). The tertiary nitrogen of procaine, N(36), is linked to O(13) of the penicillin carboxy-group. This occurs even though O(12) is slightly closer to N(36) (2.74 Å) than is O(13) (2.81 Å). The geometry is such that O(13) is located near the vacant position of the tetrahedron centred on N(36) and defined by its substituents, C(35), C(37), and C(38). Atom O(13) also accepts a hydrogen bond from O(41) of the water molecule. O(12), the other carboxy-oxygen, accepts a hydrogen bond from N(14) in the side chain of an adjoining penicillin molecule. Atom O(41) accepts hydrogen bonds from the *p*-amino-groups of two different procaine molecules to form a column along the $2_1(b)$ axis, and also donates a hydrogen bond to O(16) (the side-chain carboxy of penicillin). It is thus linked to four different molecules (two procaine and two penicillin) by hydrogen bonds, which explains its utilization in the crystal, and contributes to the relatively low solubility of this salt.

Potassium Penicillin G.—(*a*) *Penicillin G.* With one exception, bond distances and angles in KPG (Table 3) are equivalent to those in APPG. The interior angle C(5)–S(1)–C(2) at the sulphur atom is 94.7(5)°, about 3° larger than in APPG. The best four-membered plane in the thiazolidine ring is not the same as that in APPG but consists of N(4), C(5), S(1), and C(2) (χ^2 0.1) with C(3) displaced away from the β-lactam.

(*b*) *Potassium ion.* The potassium ion in KPG is co-ordinated by seven oxygen atoms (Table 4, Figure 5). All four of the oxygen atoms in penicillin G are involved.

TABLE 4

Potassium ion–oxygen distances (Å) in potassium penicillin G

$K^I \cdots O(8)$	2.726(8)	$K^I \cdots O(13^{II})$	2.850(7)
$K^I \cdots O(12^I)$	2.689(7)	$K^I \cdots O(16^I)$	2.897(8)
$K^I \cdots O(12^{II})$	2.843(7)	$K^I \cdots O(16^{II})$	2.924(10)
$K^I \cdots O(13^I)$	2.721(7)		

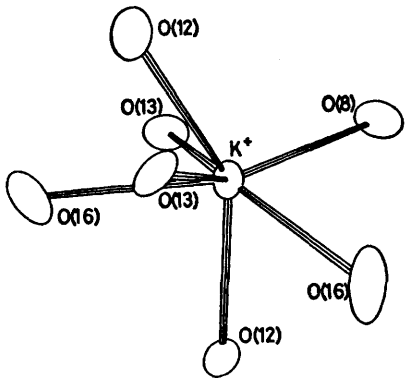

FIGURE 5 The co-ordination of the potassium ion in KPG

(*c*) *Packing.* Packing (Figure 6) in KPG is dominated by the co-ordination requirements of the potassium ion. All oxygen atoms are near a potassium ion with the result that the non-polar groups in penicillin G are on the same side of the molecule. Perpendicular to *c*, sheets of penicillin G molecules with non-polar sides back-to-back, alternate with polar sheets containing the potassium ions.

DISCUSSION

This investigation has revealed several details about the conformation adopted by the species in APPG and KPG. Quantitatively the conformational effects of different environments can best be seen in the parallel tabulation of torsion angles presented in Table 5. Considering procaine, the enlarged (91°) torsion angle

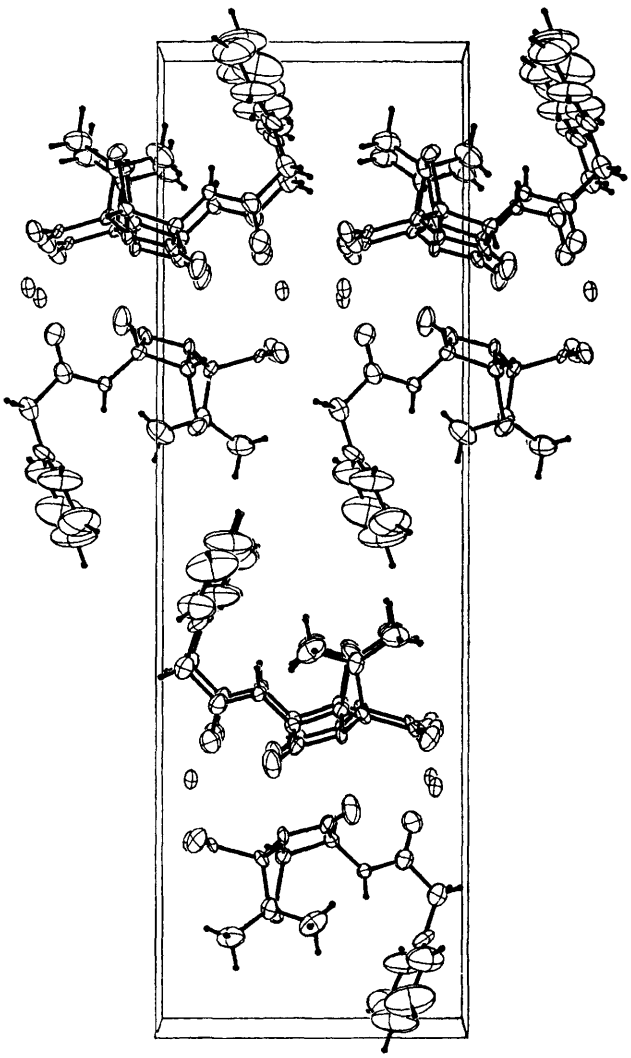

FIGURE 6 Molecular packing in the KPG crystal viewed parallel to *b*

about C(34)–C(35) is accompanied by a 60° rotation about the C(35)–N(36) bond (when compared with the hydrochloride). In APPG the two ethyl groups are thus asymmetrically placed with respect to C(34) whereas in procaine HCl they are symmetrical (Figure 6).

In penicillin, the opportunities for conformational variation are much greater. Table 5 shows that in KPG and APPG, those conformational angles which are fixed by covalent bonds are the same for the two salts; in other cases the values are different. The five-membered rings are twisted differently with, as already noted, corresponding changes in the bond angle at S(1). Further, the carboxy-groups are rotated 25° about the C(3)–C(11) bond. It is as if the attraction of the potassium ion for the carboxy-group in KPG is sufficient

to pull C(3) away from the β-lactam and thus to dictate the mode of puckering in the five-membered ring.

TABLE 5

Torsion angles (°)

(*a*) Penicillin G

Atoms	APPG	KPG	|Δ|
S(1)–C(2)–C(3)–N(4)	18.2(5)	−27.9(8)	46°
S(1)–C(2)–C(3)–C(11)	142.0(4)	94.2(8)	47
S(1)–C(5)–C(6)–N(14)	29.4(7)	15(1)	14
S(1)–C(5)–N(4)–C(7)	101.7(4)	114.3(7)	12
S(1)–C(5)–C(6)–C(7)	−90.5(5)	−109.2(8)	10
C(2)–S(1)–C(5)–C(6)	129.1(5)	97.1(8)	32
C(2)–C(3)–N(4)–C(5)	8.9(7)	32(1)	23
C(2)–C(3)–N(4)–C(7)	−108.3(6)	−86(1)	22
C(2)–C(3)–C(11)–O(12)	−105.1(5)	−79(1)	26
C(2)–C(3)–C(11)–O(13)	73.5(6)	99.5(9)	26
C(3)–N(4)–C(5)–S(1)	−31.9(6)	−19.5(8)	12
C(3)–N(4)–C(5)–C(6)	−148.0(5)	−140.0(8)	8
C(3)–N(4)–C(7)–C(6)	142.7(5)	136.9(9)	6
N(4)–C(5)–S(1)–C(2)	35.5(4)	0.1(5)	36
N(4)–C(5)–C(6)–C(7)	13.1(4)	5.4(7)	8
N(4)–C(5)–C(6)–N(14)	133.1(5)	121.2(9)	12
N(4)–C(7)–C(6)–C(5)	−13.8(4)	−5.7(7)	8
N(4)–C(7)–C(6)–N(14)	−136.4(4)	−123.5(8)	12
N(4)–C(3)–C(11)–O(13)	−165.7(5)	−141.8(8)	24
N(4)–C(3)–C(11)–O(12)	15.7(7)	40(1)	24
C(5)–S(1)–C(2)–C(3)	−31.6(4)	16.4(7)	48
C(5)–N(4)–C(7)–C(6)	14.8(4)	6.1(7)	9
C(5)–C(6)–C(7)–N(4)	−13.8(4)	−5.7(7)	8
C(5)–C(6)–N(14)–C(15)	123.0(6)	169(1)	46
C(6)–N(14)–C(15)–(C17)	169.5(5)	176(1)	6
C(7)–C(6)–N(14)–C(15)	−135.6(5)	−94(1)	42
C(9)–C(2)–C(3)–C(11)	−100.1(6)	−148.6(9)	49
C(10)–C(2)–C(3)–C(11)	24.2(6)	−26(1)	50
C(11)–C(3)–N(4)–C(5)	−115.6(5)	−91.1(9)	25
C(11)–C(3)–N(4)–C(7)	127.2(5)	149.2(9)	22
N(14)–C(15)–C(17)–C(18)	−95.6(5)	5(2)	101
C(15)–C(17)–C(18)–C(23)	−45.5(7)	−91(2)	45
C(15)–C(17)–C(18)–C(19)	136.9(6)	84(2)	53

(*b*) Procaine

	APPG	Procaine HCl	|Δ|
O(33)–C(34)–C(35)–N(36)	−91.2(6)	−70.2(3)	21
C(34)–C(35)–N(36)–C(37)	56.2(6)	68.8(3)	13
C(34)–C(35)–N(36)–C(39)	−175.9(4)	−61.1(3)	115
C(35)–N(36)–C(37)–C(38)	66.1(7)	55.3(3)	11
C(35)–N(36)–C(39)–C(40)	66.0(7)	−158.5(3)	225
C(34)–C(35)–N(36)–H(36)	−60(4)	177(2)	117

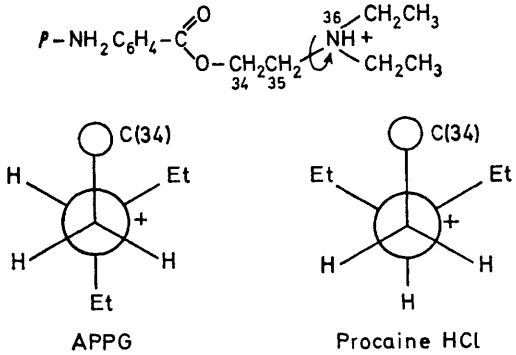

FIGURE 7 Newman projections about the C(35)–N(36) in (a) procaine HCl and (b) APPG

These observations show that the conformation of the thiazolidine ring in a particular penicillin is not fixed but is dependent on the local environment.

The largest conformational differences are found in the side-chains. Figure 8 shows the penicillin molecules in APPG and KPG in the same orientation. The side-chain is extended in APPG and is coiled in KPG, and the difference is the result of a 100° rotation about the C(15)–C(17) bond, a 50° rotation about the C(17)–C(18) bond, and a 45° rotation about the C(6)–N(14) bond.

These changes have far-reaching consequences. The array of reactive groups is changed, implying different reactivity. In KPG, not only are the nucleophilic groups on one side of the molecule and the hydrophobic groups on the other, but the amide proton [H(14)] is

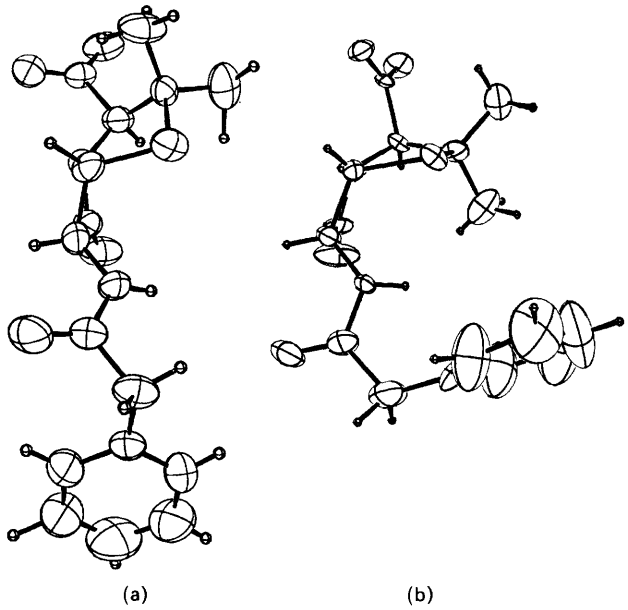

FIGURE 8 Overall molecular size and shape comparison of penicillin G in (a) procaine penicillin G and (b) potassium penicillin G. The molecules are viewed parallel to the β-lactam ring

shielded by the phenyl group and is not involved in any hydrogen bonds.

In APPG, the penicillin G molecules form head-to-tail 'helical chains' by means of hydrogen bonds between N(14) and carboxy-oxygen O(12) (Figure 4). This organization is reminiscent of cyclic peptides and it is well known that some peptides have physiological effects which far outweigh their molecular size.

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