183. Structure of Boromycin[1])

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Summary. The structure II of boromycin $C_{45}H_{74}BNO_{15}$ (*cf.* [2]) has been established by combination of chemical evidence with *X*-ray analysis. The antibiotic is a D-valine ester of a *Böeseken*-complex of boric acid with a macrodiolide of a new type.

The results of the first investigations [2] of boromycin were summarized as follows: 'a new strain of *Streptomyces antibioticus* (*Waksman et Woodruff*), ETH 28829, produces a novel antibiotic, boromycin, which appears to be the first well defined boron containing organic compound to have been found in nature. Boromycin is a complex of boric acid with a tetradentate organic complexing agent, that yields by hydrolysis D-valine, boric acid, and a polyhydroxy compound of macrolide type'.

In the meantime we have succeeded in establishing the complete structure of boromycin. We describe our results briefly in this paper and intend to publish full experimental details at a later date.

In the beginning it was attempted to elucidate the structure by chemical methods, involving mainly spectroscopic analysis of degradation products. Important indications were provided by NMR. spectra of the polyhydroxy compound obtained from boromycin by hydrolytic cleavage of D-valine and boric acid. These spectra (220 and 100 MHz) revealed the presence of 4 $CH_3C\leqslant$ and 4 $CH_3C(H)<$; a number of signals between $\delta = 2.2$ and 5 ppm indicated protons adjacent to oxygen. Acetylation of this compound yielded a pentaacetyl derivative, whose NMR. spectra displayed signals of 5 distinct acetyl groups. Many attempts to degrade the polyhydroxy compound by hydrolysis or oxidation led to mixtures from which pure products could not be isolated. Finally, a crystalline 'diol' was obtained by oxidation of the only C=C double bond present by osmium(VIII)-oxide in *t*-butanol. This 'diol' yielded by periodate oxidation three well defined products: 1. acetaldehyde (isolated as its 2,4-dinitrophenylhydrazone), 2. an acid, $C_{13}H_{24}O_4$, and 3. a neutral compound, $C_{18}H_{32}O_5$. Spectroscopic analysis (NMR., IR., MS.) revealed many structural details of 2. and 3., but it was not possible to deduce unambiguous constitutions for them. In particular, these compounds contained so many features in common that it was not possible to decide whether they arose from the same part of the original molecule or from different parts. At this stage, before carrying out further degradation experiments, it seemed most reasonable to go over to *X*-ray analysis.

A crystalline Cs salt (corresponding to the Na salt described in [2]) was obtained by hydrolytic cleavage of D-valine from boromycin by caesium hydroxide. Recrystallization from warm aqueous methanol produced thick orthorhombic prisms ($a = 12.70$,

[1]) 95th Comm. of the series Metabolic Products of Microorganisms. – 94th comm.: [1].

$b = 17.61$, $c = 21.69$ Å, space group $P2_12_12_1$, $Z = 4$). For *X*-ray analysis the crystals had to be sealed in glass capillaries, since they lost solvent of crystallization and became opaque on exposure to air. The *X*-ray diffraction pattern, even of sealed translucent crystals, both at room temperature and at $-130°$, faded rapidly with increasing scattering angle and became virtually unobservable at $\sin\Theta/\lambda$ values of 0.35–0.40, indicating unusually large thermal motion or disorder in the molecular arrangement within the crystals. This limitation in the amount of data observable was obviously going to lead to difficulties associated with inadequate resolution of the electron density distribution. However, since attempts to produce more suitable crystals were unsuccessful at that time we decided to proceed with the structure analysis of these unpromising crystals.

A first attempt at structure determination was based on a data set of 945 independent F values extending to $\sin\Theta/\lambda = 0.34$ (intensity measurements with 4-circle diffractometer, Mo$K\alpha$ radiation). The Cs atom was located from the *Patterson* function, and several *Fourier* syntheses, phased first on Cs alone and then on various partial structures, were calculated, but all attempts to draw any firm conclusions concerning the molecular structure were frustrated by inadequate resolution.

In principle, measurement of *Friedel* pairs of reflexions can lead to a direct solution of the phase problem for a non-centrosymmetric crystal containing an anomalous scatterer [3]. Intensities of both hkl and $\bar{h}\bar{k}\bar{l}$ reflexions were measured from a somewhat larger crystal to $\sin\Theta/\lambda \sim 0.40$ (2173 reflexions in all) using Cu$K\alpha$ radiation in order to excite the anomalous scattering of the caesium atom ($\Delta f' = -1.7$ e, $\Delta f'' = 8.3$ e). In the space group $P2_12_12_1$ the set of 4 caesium atoms in general positions is itself chiral, but phase angles derived from the two enantiomorphic arrangements, $x = (x,y,z)$ and $\bar{x} = (\bar{x},\bar{y},\bar{z})$, by making use of the observed *Bijvoet* differences, lead to electron-density distributions that are not enantiomorphic, *i.e.* $\alpha_H(x) \neq -\alpha_H(\bar{x})$. The density distribution based on the Cs coordinates (0.140, −0.069, 0.179) was completely uninterpretable; the one based on the enantiomorphic coordinates (0.140, 0.069, 0.179) had many features in common with previously calculated distributions phased on partial structure models, and several chemically plausible fragments could be recognized as defining the rough overall shape of the molecule as seen at low resolution. We were thus in the unusual position of having determined the absolute configuration of the molecule without knowing its structural formula!

A series of *Fourier* syntheses, phased initially only on selected peaks and subsequently on increasingly larger molecular fragments as they were recognized, led gradually to the approximate location of 53 atoms in the molecular skeleton, together with a molecule each of methanol and water. Several structural features had to be inferred from stereochemical arguments since experimental evidence for them was tenuous. In particular, no significant peak was found at the expected site of the boron atom – the centre of an approximately regular tetrahedron of oxygen atoms. Moreover, although most of the oxygen atoms could be recognized by their higher electron density, some were identified as such merely from their stereochemical environments. Least-squares refinement of the structure model led to an R factor of 0.13, based on approximately 1300 independent reflexions, but the isotropic B values of several atoms oscillated wildly from cycle to cycle so that we were not convinced of the correctness of the structure model.

Among other things, it was by no means clear how the degradation products *2.* and *3.* could have arisen from the tentative structure, which later proved to be incorrect in the region between C(9′) and C(12′). This region of the electron-density distribution was so ill defined that any interpretation of its rather weak and diffuse features had to be regarded with some scepticism. It is quite likely that the poor definition in this region is associated with torsional oscillations of large amplitude in this part of the molecule in the orthorhombic crystal modification described above.

At this stage renewed attempts were made to prepare more suitable crystals of the Cs as well as of the Rb salt. It was found that crystallization from aqueous methanol now yielded monoclinic crystals (the orthorhombic form could no longer be reproduced!). The monoclinic crystals of the Cs and Rb salts are isomorphous (Cs salt: $a \sim 12$, $b \sim 18$, $c \sim 12$ Å, $\beta \sim 111°$; Rb salt: $a = 11.98$, $b = 18.31$, $c = 11.99$ Å, $\beta = 111.33°$, space group $P2_1$, $Z = 2$) and give *X*-ray diffraction patterns containing more than three times as many reflexions (extending to $\sin\Theta/\lambda \sim 0.6$) as the orthorhombic crystals. Intensities of 3232 independent reflexions from the Rb salt (sealed in a glass capillary) were measured with a four-circle diffractometer (Mo$K\alpha$ radiation).

In the space group $P2_1$ any two symmetry-equivalent atoms are related by the symmetry elements of the higher space group $P2_1/m$ so that the *Fourier* synthesis phased on Rb contributions alone also has this higher symmetry. However, armed with a fairly detailed structural model and aided by the fortuitous location of the molecule with respect to the false mirror plane, we encountered little difficulty in

Compound I as Rb salt: Positional and thermal parameters (standard deviations in units of least significant digit)
For atom-numbering see formula I

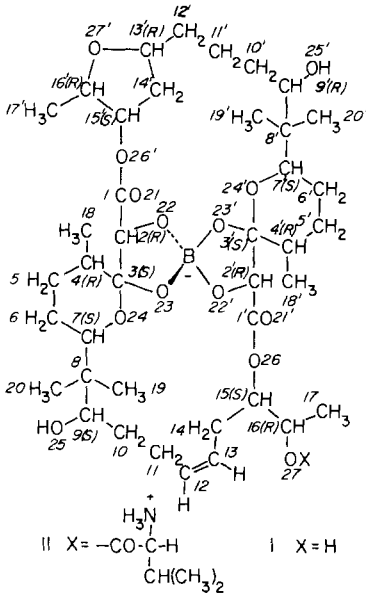
Atom	x	y	z	B(Å²)	Atom	x	y	z	B(Å²)
C(1)	0.3649(10)	0.0905(7)	0.0241(10)	3.9(2)	C(4′)	0.0060(12)	0.0374(8)	0.3774(12)	4.9(3)
C(2)	0.3305(9)	0.0624(6)	0.1243(9)	3.2(2)	C(5′)	-0.0724(14)	-0.0283(9)	0.3091(14)	6.3(3)
C(3)	0.4239(9)	0.0761(6)	0.2489(9)	3.6(2)	C(6′)	-0.1579(14)	-0.0074(9)	0.1956(15)	7.2(4)
C(4)	0.5258(11)	0.0182(7)	0.2902(11)	4.7(3)	C(7′)	-0.0993(12)	0.0324(7)	0.1170(12)	4.8(3)
C(5)	0.6248(14)	0.0452(9)	0.3987(14)	6.9(4)	C(8′)	-0.1813(11)	0.0584(7)	-0.0080(12)	4.7(3)
C(6)	0.6666(13)	0.1189(8)	0.3826(13)	6.2(3)	C(9′)	-0.1017(12)	0.1016(8)	-0.0613(12)	5.0(3)
C(7)	0.5619(12)	0.1736(8)	0.3485(12)	4.6(3)	C(10′)	-0.1524(15)	0.1123(9)	-0.1968(15)	6.4(4)
C(8)	0.5873(11)	0.2521(11)	0.3181(11)	5.3(3)	C(11′)	-0.0795(16)	0.1601(10)	-0.2430(15)	6.3(4)
C(9)	0.4731(12)	0.3002(8)	0.2884(12)	5.2(3)	C(12′)	0.0437(14)	0.1288(9)	-0.2230(13)	5.7(3)
C(10)	0.4145(13)	0.2986(8)	0.3806(13)	4.9(3)	C(13′)	0.1263(13)	0.1759(9)	-0.2629(13)	6.2(3)
C(11)	0.3202(12)	0.3580(8)	0.3594(11)	5.2(3)	C(14′)	0.2503(13)	0.1385(8)	-0.2358(13)	6.0(3)
C(12)	0.2551(13)	0.3537(8)	0.4448(12)	5.1(3)	C(15′)	0.3335(12)	0.1798(8)	-0.1279(12)	4.8(3)
C(13)	0.1396(12)	0.3660(8)	0.4220(11)	5.3(3)	C(16′)	0.2795(12)	0.2564(11)	-0.1458(11)	6.3(3)
C(14)	0.0500(12)	0.3892(8)	0.3022(12)	5.1(3)	C(17′)	0.3227(18)	0.3014(12)	-0.2334(18)	9.5(5)
C(15)	-0.0447(11)	0.3312(7)	0.2427(11)	4.4(2)	C(18′)	0.1043(12)	0.0119(8)	0.4902(12)	5.9(3)
C(16)	-0.1197(11)	0.3483(7)	0.1152(11)	4.9(3)	C(19′)	-0.2892(14)	0.1048(9)	-0.0036(13)	7.3(3)
C(17)	-0.2012(14)	0.4145(9)	0.1079(14)	6.7(4)	C(20′)	-0.2295(15)	-0.0130(9)	-0.0846(15)	7.7(4)
C(18)	0.4810(13)	-0.0564(9)	0.3135(13)	6.2(3)	O(21′)	-0.0328(7)	0.2186(5)	0.3940(8)	4.9(2)
C(19)	0.6346(11)	0.2575(11)	0.2173(12)	6.5(3)	O(22′)	0.1903(6)	0.1663(4)	0.2799(6)	3.1(1)
C(20)	0.6869(14)	0.2846(9)	0.4341(14)	7.3(4)	O(23′)	0.1469(6)	0.0418(4)	0.2733(6)	3.5(1)
O(21)	0.4314(8)	0.0565(5)	-0.1223(8)	5.4(2)	O(24′)	-0.0415(7)	0.0965(4)	0.1866(7)	4.0(2)
O(22)	0.2234(6)	0.0962(4)	0.1274(6)	3.3(1)	O(25′)	-0.0792(8)	0.1725(5)	-0.0094(8)	5.6(2)
O(23)	0.3357(6)	0.0776(4)	0.3220(6)	3.5(1)	O(26′)	0.3159(7)	0.1538(5)	-0.0195(7)	4.3(2)
O(24)	0.4715(6)	0.1459(4)	0.2407(6)	3.8(1)	O(27′)	0.1569(8)	0.2431(6)	-0.1898(8)	6.2(2)
O(25)	0.3878(7)	0.2817(4)	0.1727(7)	4.6(2)	B	0.2291(11)	0.0959(7)	0.2484(11)	3.4(2)
O(26)	0.0176(5)	0.2622(5)	0.2450(5)	3.6(1)	Rb^+	0.1365(1)	0.2500(0)	0.0581(1)	*)
O(27)	-0.0426(8)	0.3597(5)	0.0512(8)	5.6(2)	C(S1)	0.4505(30)	0.0379(20)	0.6232(30)	11.3(7)
C(1′)	0.0265(10)	0.2139(6)	0.3313(10)	3.6(2)	C(S2)	-0.1947(12)	0.3549(8)	-0.2651(11)	8.6(3)
C(2′)	0.1119(10)	0.1538(6)	0.3429(10)	3.3(2)	O(S1)	0.3966(20)	0.1032(13)	0.5638(20)	9.9(5)
C(3′)	0.0547(10)	0.0791(7)	0.2944(10)	3.6(2)	O(S2)	-0.1611(9)	0.4096(5)	-0.1748(8)	7.2(2)

*) For the Rb atom we employed an anisotropic temperature factor of the form:
$T = \exp[-(b_{11}h^2 + b_{22}k^2 + b_{33}l^2 + b_{12}hk + b_{13}hl + b_{23}kl)]$ with coefficients
$b_{11} = 0.0086$, $b_{22} = 0.0039$, $b_{33} = 0.0080$, $b_{12} = 0\ 0015$, $b_{13} = 0.0072$, $b_{23} = 0.0009$.

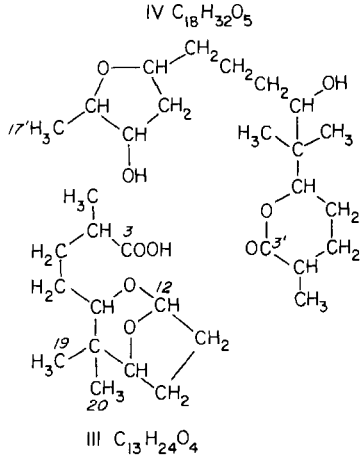
interpreting the Rb-phased *Fourier* synthesis. Two subsequent electron-density syntheses, phased on models from which some atoms had been purposely omitted for control purposes, led unequivocally to structure I. Least-squares refinement (hydrogen atom contributions included; isotropic B values for all atoms except Rb) led to convergence at $R = 0.06$. In the final least-squares cycle all parameter shifts were less than their respective standard deviations. A difference synthesis revealed no residual electron-density greater than 0.5 e Å^{-3}. Final positional and thermal parameters for Rb, B, C and O atoms, and two CH_3OH of crystallization, are listed in the Table.

Estimated standard deviations of the positional parameters of the B, C and O atoms lie in the range 0.007–0.019 Å. Bond distances and angles derived from the coordinates are in reasonable agreement with values expected for I; they lie in the following ranges: C–C (sp^3, sp^3), 1.48–1.58 Å; C–C (sp^3, sp^2), 1.48–1.51 Å; C=C (sp^2, sp^2), 1.33 Å; C=O, 1.21 Å; C(O)–O, 1.31–1.34 Å; C(O)O–C, 1.42–1.47 Å; C–OH, 1.42–1.43 Å; B–O, 1.43–1.50 Å.

The overall shape of the anion I (both in the monoclinic and orthorhombic crystals) is roughly that of a sphere with a lipophilic surface and a cleft lined with oxygen atoms. The cation is housed in this cleft, coordinated by 6 to 8 oxygens in an irregular arrangement. Chemically the anion I is a *Böeseken* complex of boric acid with a macrodiolide consisting of two almost identical halves. The only constitutional difference is that instead of the tetrahydrofurane ring in one half there is a hydroxyl and a *cis* double bond in the other. The other remarkable difference is that the configurations at C(9) and C(9′) are opposite, whereas those of all other corresponding pairs are the same.



II X = –CO–C(H)(NH$_3^+$)–CH(CH$_3$)$_2$ I X = H



IV $C_{18}H_{32}O_5$

III $C_{13}H_{24}O_4$

The structure of boromycin itself, II, can be derived from I with confidence by inspection of space-filling models, assuming that the observed, evidently rigid con-

formation of the anion remains essentially unchanged in the antibiotic. Of the three hydroxyls present in the anion only O(27) can be esterified by D-valine in such a way that the $-NH_3^+$ can be accommodated within the oxygen-lined cleft, which is thereby blocked by the lipophilic part of the amino acid.

The result of the *X*-ray analysis requires that the previous empirical formula of I, $C_{39}H_{64}BO_{15}X$ [2] has to be amended to $C_{40}H_{64}BO_{14}X$. The empirical formula of boromycin itself is $C_{45}H_{74}BNO_{15}$ (instead of $C_{44}H_{72}BNO_{15}$ [2]) and that of the B- and N-free hydrolysis product $C_{40}H_{68}O_{14}$ (instead of $C_{39}H_{66}O_{14}$ [2]).

Structures III and IV can now be assigned to the degradation products $C_{13}H_{24}O_4$ and $C_{18}H_{32}O_5$, which have evidently originated from different halves of the molecule. The IR., NMR., and MS. are in full agreement with these structures. Acetaldehyde, also formed in the degradation, is derived from the side chain C(16)–C(17) but could also be formed by hydrolysis of malonic dialdehyde, a likely primary degradation product of the fragment C(13)–C(14)–C(15).

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184. Synthèse du méthanesulfonate de (pyrimidyl-2)-1-(méthylène)-(dioxy-3,4-benzyl)-4-pipérazinium (Piribedil)[1]) marqué au ^{14}C

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Summary. The synthesis of crystalline 1-(2-pyrimidyl)-4-(3,4-methylenedioxybenzyl)-piperazin methanesulfonate (ET 495) labelled with ^{14}C fixed on the piperazine nitrogen N(4) has been realized in five steps from barium carbonate ^{14}C; Specific activity of the final product: 32,8 mCi/mmol (110 μCi/mg); radiochemical purity: around 99%. The mass spectrum of the labelled compound is in agreement with the chemical structure and the labelling position expected.

De nombreuses aralkyl-1-pipérazines substituées en 4 sont connues pour leur activité hypotensive et vasodilatatrice. Une nouvelle série, les pipéronylpipérazines, a été synthétisée par *Regnier, Canevari, Laubie & Le Douarec* [1]. Dans cette série, la

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