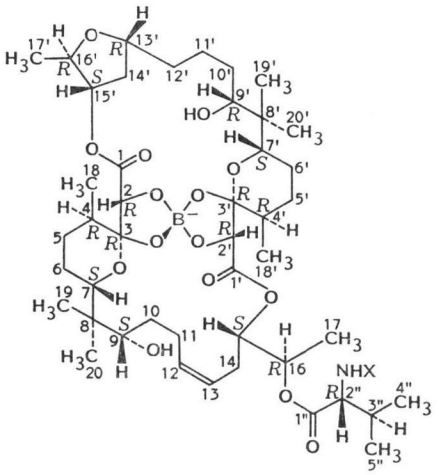
ISOLATION OF TWO MINOR COMPONENTS OF THE BOROMYCIN FERMENTATION: *N*-ACETYLBOROMYCIN AND *N*-FORMYLBOROMYCIN

Sir:

Boromycin (**I**), a metabolite of *Streptomyces antibioticus*[1~3], is the first member of a small group of macrodiolide antibiotics containing boron as a center atom. Other members are aplasmomycin[4] and its mono- and di-*O*-acetate[5]. In the course of our biosynthetic studies on boromycin[6] we had occasion to examine chromatographically boromycin samples of different origin, obtained from Professor PRELOG, from Ciba-Geigy, from Merck Sharp and Dohme, and samples isolated in our own laboratories from *Streptomyces* sp. MA 4423[3]. We



Boromycin (**I**) X=H
N-Formylboromycin (**II**) X=CHO
N-Acetylboromycin (**III**) X=$COCH_3$

Table 1. The ^1H chemical shifts and ^1H-^1H spin coupling constants for *N*-formyl- and *N*-acetylboromycin.

Assignment	Chemical shift (ppm),[a] coupling constant (Hz)	
	N-Formylboromycin	*N*-Acetylboromycin
H-2, H-2′	4.43 (s), 4.42 (s)	4.43 (s), 4.42 (s)
H-7	4.09 (dd, 7.5, 6.5)	4.08 (d, 7.3, 6.3)
H-7′	3.85 (bd, 10.6)	3.86 (dd, 11.5, 1.3)
H-9	2.95 (dd, 10.2, 7.2)	2.95 (dd, 10.1, 7.1)
H-9′	3.66 (dd, 11.9, 2.9)	3.67 (dd, 11.9, 2.6)
9-OH	5.16 (d, 7.0)	5.1 (d, 7.0)
9′-OH	6.07 (s)	6.0 (s)
H-11*R*	2.58 (m)	2.57 (dt, 13.6, 10.4)
H-12	5.49 (t, 10.3)	5.5 (t, 10.4)
H-13	5.19 (bt, 10.3)	5.19 (bt, 10.4)
H-13′	4.13 (t, 10)	4.16 (t, 10)
H-14*R*	2.49 (dt, 13.5, 10.4)	2.45 (dt, 13.4, 10.4)
H-14′*R*	2.49 (ddd, 14.8, 10, 3.5)	2.48 (ddd, 14.7, 10, 3.75)
H-14′*S*	1.88 (d, 14.8)	1.88 (d, 14.7)
H-15	5.31 (dd, 12.4, 2.9)	5.31 (bd, 13.6)
H-15′	5.0 (d, 3.5)	5.0 (d, 3.74)
H-16	5.27 (q, 7.2)	5.3 (q, 7.1)
H-16′	4.99 (q, 6.6)	4.97 (q, 6.7)
H-17	1.39 (d, 7.1)	1.38 (d, 7.1)
H-17′	1.13 (d, 6.6)	1.14 (d, 6.7)
H-18, H-18′	1.04 (d, 7.1), 1.03 (d, 7.1)	1.04 (d, 7.2), 1.03 (d, 7.2)
H-19	0.69 (s)	0.69 (s)
H-19′	0.58 (s)	0.59 (s)
H-20	0.93 (s)	0.92 (s)
H-20′	0.65 (s)	0.66 (s)
–NH	7.98 (d, 8.4)	7.48 (d, 8.7)
H-2″	4.71 (dd, 8.4, 5.1)	4.68 (dd, 8.7, 5.6)
H-3″	2.15 (qq)	2.17 (qq)
–CHO	8.14 (s)	$-COCH_3$; 2.0 (s)

[a] Spectra were recorded in $CDCl_3$; chemical shifts are given relative to TMS.

noted that all these samples contained a minor component of very similar behavior to boromycin.

To isolate the minor component, the ethyl acetate extract of mycelia of *Streptomyces* sp. MA 4423, cultivated as described[6], was subjected to column chromatography (silica gel 230~400 mesh, $CHCl_3$ - CH_3OH, 95: 5, **I**, Rf 0.38, unknown 0.50, and benzene - EtOAc, 1: 1, **I** Rf 0.51, unknown 0.68, recovery **I** 10 mg/liter, unknown 1.2 mg/liter). ^1H NMR of the material, which at this point appeared homogeneous in the above TLC systems, indicated that it consisted of a 1: 3 mixture of two compounds, which could subsequently be separated by TLC (silica gel, hexane - acetone, 3: 1). Positive ion FAB mass spectrometry gave monoisotopic molecular weights of 907.5 (compound **II**, $C_{46}H_{74}BNO_{16}$-(Na)) and 921.5 (compound **III**, $C_{47}H_{76}BNO_{16}$-(Na)), respectively, indicating a mass difference of 28 and 42 from **I**[7]. The ^1H NMR spectra (Nicolet NT-500, $CDCl_3$) of both compounds (Table 1) closely resembled the spectrum of boromycin[8,9], but some small, diagnostic differences were apparent. The NH proton in both compounds appeared as a doublet, indicating monosubstitution of the nitrogen. In agreement with this conclusion, neither compound gave a positive ninhydrin reaction, although both formed valine upon hydrolysis. The presence of a singlet at 8.14 ppm in the spectrum of **II**, assigned to a formyl group, together with the molecular weight suggests that **II** is *N*-formylboromycin. Similarly, an acetyl methyl group (2.0 ppm) in the spectrum of **III** suggests *N*-acetylboromycin as the structure of this compound.

To confirm these tentative structural assignments we prepared authentic samples of **II** and **III** from boromycin. **III** was obtained by stirring 20 mg **I** with 0.5 ml acetic anhydride for 3 hours at room temp, followed by evaporation and preparative TLC (yield 14 mg, 67%, white powder, mp 250~255°C (dec), UV end absorption, $[\alpha]_D^{22}$ +65.1° (*c* 0.175, $CHCl_3$)). To prepare **II**, an ice-cold mixture of 80 mg dicyclohexylcarbodiimide and 46 mg HCOOH in chloroform was added to 18 mg **I** in 0.5 ml pyridine and the reaction was stirred for 3 hours in an ice bath. Filtration of the dicyclohexylurea, evaporation of the pyridine and preparative TLC of the residue gave 17 mg **II** (yield 93.6%, white powder, mp 273~277°C (dec), UV end absorption, $[\alpha]_D^{22}$ +63.0° (*c* 0.270, $CHCl_3$)). The synthetic samples of **II** and **III** agreed in all respects (MS, ^1H NMR, co-chromatography in several solvent systems) with the isolated materials.

The two minor constituents of the boromycin fermentation described here differ from those of the aplasmomycin fermentation in that acylation has occurred not on the 9-hydroxyl group but on the nitrogen of the valine moiety, a structural feature not present in aplasmomycin. Interestingly, as the close similarity of the ^1H NMR data for **I**, **II**, and **III** indicates, acylation of the nitrogen with the consequent elimination of the charge on the nitrogen has little or no effect on the solution conformation of the molecule.

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