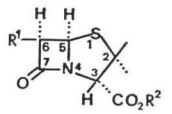
## Communications to the editor

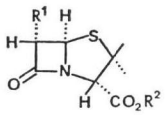
# 6β-HALOPENICILLANIC ACIDS, A GROUP OF β-LACTAMASE INHIBITORS

Sir:

Recent reports have described the preparation of a novel β-lactamase inhibitor, 6β-bromopenicillanic acid (**1a**)[1~4], obtained as a mixture with its inactive 6α-bromo epimer **2a**[5], either by aqueous equilibration of the latter at pH 9.1 and 30°C for 3~4 days or by selective reduction of 6,6-dibromopenicillanic acid (**3**)[6]. However, the isolation of pure **1a** from such mixtures has not been previously recorded. The present communication describes the isolation of 6β-bromopenicillanic acid (**1a**) as well as the analogous 6β-chloro and 6β-iodo compounds **4a** and **5a** by separation of mixtures with the corresponding 6α-halopenicillanic acids from which they were prepared*.

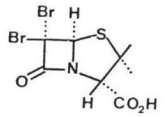
Incubation of 0.1 M aqueous potassium 6α-bromopenicillanate (**2b**) (300 ml) at pH 9.0 (pH-stat) and 30°C for 24 hours provided a mixture of **1b** and **2b** in an approximate ratio of 25: 75 as shown by $^1$H-NMR spectroscopy. After addition of diethyl ether (200 ml) and acidification (pH 3) of the stirred mixture at 5°C with 4 N hydrochloric acid, the resulting organic extract was dried and concentrated at reduced pressure to about 40 ml. The concentrate was subjected to dry-column chromatography on silica gel (Silica Woelm TSC, 600 g), the column (quartz glass, 6×50 cm) being treated with 100 ml of developing solvent (diethyl ether - petroleum ether - formic acid, 70: 30: 0.1) before loading. Separation of the epimeric mixture was accomplished with 1,200 ml of developing solvent, the major part of the 6α-bromo epimer **2a** being eluted into the outflow. From the bottom of the column, segments (2 cm each) were scraped out, suspended in ethyl acetate (10 ml), and



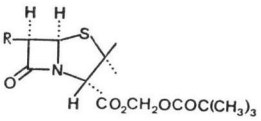


**1a** $R^1$=Br, $R^2$=H
**1b** $R^1$=Br, $R^2$=K
**4a** $R^1$=Cl, $R^2$=H
**4b** $R^1$=Cl, $R^2$=K
**5a** $R^1$=I, $R^2$=H
**5b** $R^1$=I, $R^2$=K

**2a** $R^1$=Br, $R^2$=H
**2b** $R^1$=Br, $R^2$=K
**6a** $R^1$=Cl, $R^2$=H
**6b** $R^1$=Cl, $R^2$=K
**7a** $R^1$=I, $R^2$=H
**7b** $R^1$=I, $R^2$=K
**8** $R^1$=I, $R^2$=$CH_2OCOCH_3$



**3**



**9** R=Br, **10** R=Cl, **11** R=I

aliquots (5 μl) of the supernatants were examined by thin-layer chromatography (Silica Gel 60 F-254, Merck) using the above developing solvent (2 runs). Fractions containing the pure 6β-bromo epimer **1a** were combined and eluted with ethyl acetate. The resulting extract was concentrated *in vacuo* to about 40 ml, washed thoroughly with water to remove formic acid, and dried. Further evaporation of the concentrate to about 5 ml afforded precipitation of crystalline 6β-bromopenicillanic acid (**1a**) which, after recrystallization from diethyl ether, decomposed at 80~100°C without melting; $[\alpha]_D^{20}$ +272° (*c* 0.5, $CHCl_3$).

Similarly, equilibration (pH 9.0~9.2, 30°C,

Table 1. $^1$H-NMR spectra of 6β-halopenicillanic acids in $CD_3CN$. Signals listed in δ values using TMS as internal reference.

| Position of protons | Chemical shift (δ) | | |
|---|---|---|---|
| | **1a** | **4a** | **5a** |
| 2α-$CH_3$ | 1.51 s | 1.51 s | 1.49 s |
| 2β-$CH_3$ | 1.63 s | 1.61 s | 1.65 s |
| 3-H | 4.46 s | 4.45 s | 4.45 s |
| 5-H | 5.48 d J=4.0 | 5.38 d J=4.0 | 5.35 d J=4.0 |
| 6-H | 5.54 d J=4.0 | 5.58 d J=4.0 | 5.74 d J=4.0 |

s=singlet, d=doublet, J=coupling constant in Hz.

* Subsequent to the completion of this work a report describing the preparation of pure sodium 6β-bromopenicillanate through stereoselective reduction of trimethylsilyl 6,6-dibromopenicillanate with tri-*n*-butyltin hydride followed by hydrolysis and sodium salt formation has appeared.[7]

24 hours) of 0.1 M aqueous solutions of potassium 6α-chloropenicillanate (**6b**)[5] and the corresponding 6α-iodo derivative **7b** (obtained after hydrolysis and salt formation from the acetoxymethyl ester **8** prepared in analogy to a published method[8]) provided epimeric mixtures containing 10~15 and 30~35%, respectively, of the 6β-halopenicillanates **4b** and **5b** as indicated by $^1$H-NMR spectroscopy. After extraction of the acidified mixtures (pH 3) with diethyl ether, the 6β-halo acids (**4a**, **5a**) were separated from the respective 6-epimers (**6a**, **7a**) by dry-column chromatography on silica gel using the same procedure as described above. On concentration of the eluates, the pure 6β-epimers crystallized from ethyl acetate to afford, after recrystallization from diethyl ether-diisopropyl ether, **4a**, $[\alpha]_D^{20}$ +264° (*c* 0.5, $CHCl_3$); and **5a**, $[\alpha]_D^{20}$ +276° (*c* 0.5, $CHCl_3$). Both compounds decomposed at 80~100°C without melting.

As apparent from the $^1$H-NMR data (Table 1), the β-lactam protons in **1a**, **4a**, and **5a** have a larger coupling constant (J=4.0 Hz) than that observed in 6α-halopenicillanates (J=1.5 Hz)[6,9,10], and therefore the 6β-halo compounds contain a *cis*-oriented β-lactam and have the 5*R*, 6*R*-configuration.

Alternatively, the 6β-halo acids could be isolated, after salt formation with 1 M aqueous potassium bicarbonate and removal of water by azeotropic distillation with *n*-butanol, as the crystalline potassium salts **1b**, $[\alpha]_D^{20}$ +253° (*c* 0.5, 1 M phosphate buffer pH 7); **4b**, $[\alpha]_D^{20}$ +243° (*c* 0.5, 1 M phosphate buffer pH 7); and **5b**, $[\alpha]_D^{20}$ +260° (*c* 0.5, 1 M phosphate buffer pH 7).

The 6β-halopenicillanic acids were further characterized as the corresponding pivaloyloxymethyl esters **9**, m.p. 67~68°C, $[\alpha]_D^{20}$ +197° (*c* 0.5, $CHCl_3$); **10**, m.p. 68~69°C, $[\alpha]_D^{20}$ +185° (*c* 0.5, $CHCl_3$); and **11**, m.p. 78~79°C, $[\alpha]_D^{20}$ +201° (*c* 0.5, $CHCl_3$), obtained from the potassium salts **1b**, **4b**, and **5b** on treatment with chloromethyl pivalate in dimethylformamide.

The 6β-halopenicillanic acids exhibit only weak antibacterial activity against most Gram-positive and Gram-negative bacteria (with the exception of the genus *Neisseria*), but are potent inhibitors, in particular **1a** and **5a**, of various types of β-lactamases. Thus, the inhibitory activities of 6β-bromopenicillanic acid (**1a**) and its iodo analog **5a**, *e.g.* against the enzymes from *Staphylococcus aureus* and *Escherichia coli* (RTEM), compare favorably with that of clavulanic acid, whereas 6β-chloropenicillanic acid (**4a**) is less active*.

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* Personal communication from Dr. BODIL BALTZER.