# PLATOMYCINS A AND B

## II. PHYSICOCHEMICAL PROPERTIES

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(Received for publication May 6, 1975)

Platomycins A and B, two new antibacterial and antitumor antibiotics were found to belong to the phleomycin-bleomycin family, being closely related to bleomycins B. The two ahtibiotics have been differentiated from all of the reported phleomycins and bleomycins.

During the course of the screening for new antibiotics, a mixture of antibacterial and antitumor antibiotics was isolated from the fermentation broth of *Streptosporangium violacechromogenes* subsp. *globophilum*. The mixture was separated into two active components, platomycins A and B.

Taxonomic studies of the producing strain, as well as the production, isolation and biological properties of the platomycins were described in a previous paper.[1]

This communication deals with the physicochemical properties and the results of preliminary degradation studies on these antibiotics.

### Physicochemical Properties of Platomycins

Platomycins A This antibiotic was isolated as a blue-colored amorphous hydrochloride, highly soluble in water, soluble in methanol, slightly soluble in ethanol and insoluble in such organic solvents as higher alcohols, acetone, chloroform, ethylacetate, butylacetate, ethylether and benzene. Platomycin A decomposed above 220°C. The elemental analysis was; C, 40.12%; N, 15.38%; H, 5.75%; S, 2.08%; Cl, 7.85% and Cu, 3.90%. Platomycin A showed UV maxima at 244 nm ($E^{1\%}_{1cm}$ 110) and 293 nm ($E^{1\%}_{1cm}$ 84). The ratio of the intensity of the absorption at 244 nm to that at 293 nm was 1.31 (Fig. 1). The IR spectrum of this compound in KBr tablet is shown in Fig. 2. Platomycin A gave positive SAKAGUCHI, PAULI and EHRLICH reactions and negative ninhydrin reaction. The optical rotatory dispersion (ORD) curve of platomycin A (*c* 0.5, water) shows a positive COTTON effect at 558 nm peak $[\alpha]^{30}_{610}+137.6$, D-line $[\alpha]^{30}_{D}+100$ and trough $[\alpha]^{30}_{510}-88$. Platomycin A was stable in acidic and neutral solution but somewhat unstable in alkaline solution (Table 1).

Fig. 1. UV Spectra of the platomycins in aqueous solution.

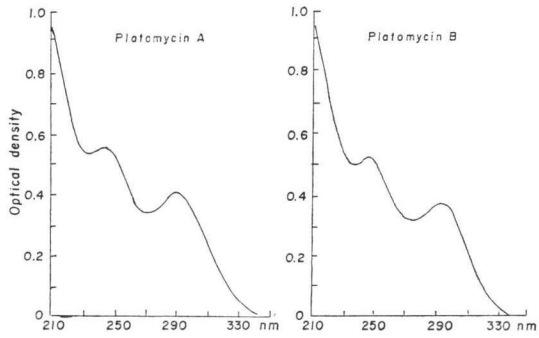


Fig. 2. IR spectrum of platomycin A in KBr tablet.

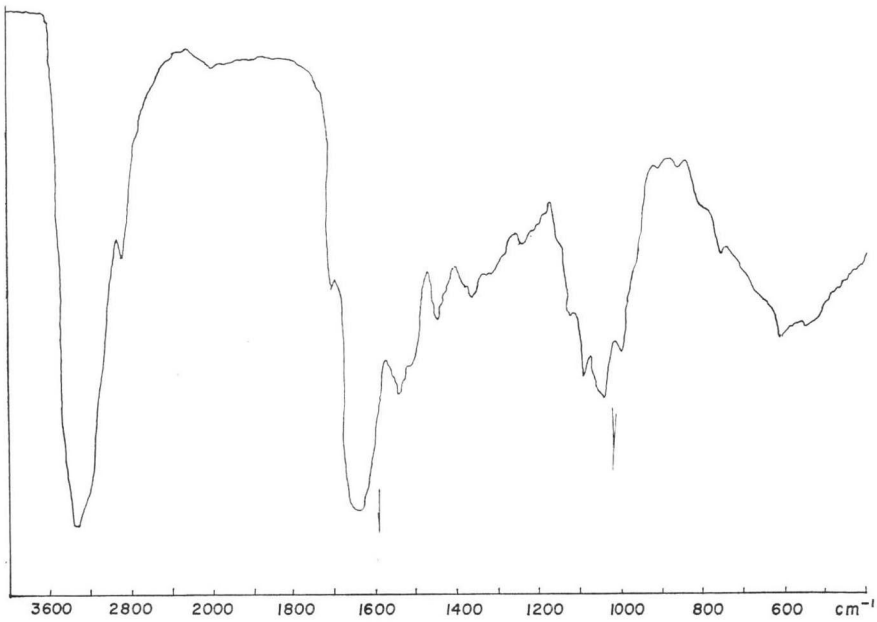
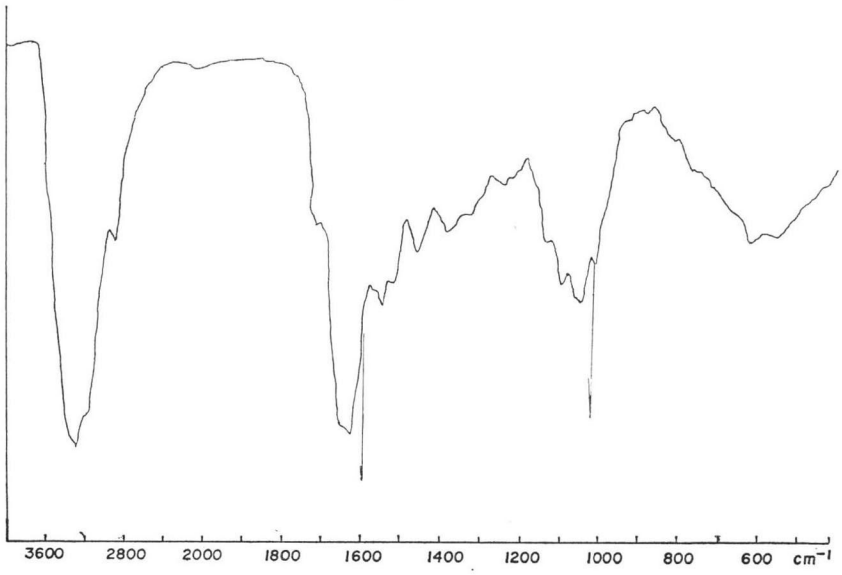



Fig. 3. IR spectrum of platomycin B in KBr tablet.




Platomycin B This antibiotic was also isolated as a blue-colored and amorphous hydrochloride. Its solubility, color reaction and stability (Table 1) were almost the same as those of platomycin A. Platomycin B decomposed above 200°C. The elemental analysis was: C, 40.04 %; N, 15.80 %; H, 5.89 %; S, 2.65 %; Cl, 7.39 % and Cu, 5.35 %. As seen in Fig. 1, platomycin B showed UV maxima at 244 nm ($E^{1\%}_{1cm}$ 102) and 293 nm ($E^{1\%}_{1cm}$ 83). The ratio of the intensity of the absorption at 244 nm to that at 293 nm was 1.23 (Fig. 1). The IR spectrum of this compound in KBr tablet is shown in Fig. 3. The ORD curve of platomycin B (*c* 0.5, water) shows

a positive COTTON effect at 558 nm, peak $[\alpha]_{612}^{30}+159.2$, D-line $[\alpha]_{D}^{30}+112.0$ and trough $[\alpha]_{506}^{30}-112.0$.

Table 1. Stability of the platomycins in aqueous solution

| Temp. | pH | Activity remaining (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Platomycin A | | | | Platomycin B | | | |
| | | 0 | 30 min. | 2 hrs. | 4 hrs. | 0 | 30 min. | 2 hrs. | 4 hrs. |
| 30°C | 2.5 | 100 | 100 | 100 | 100 | 100 | 100 | 96 | 96 |
| | 7.0 | 100 | 100 | 90 | 100 | 100 | 86 | 100 | 100 |
| | 9.5 | 100 | 97 | 97 | 60 | 105 | 105 | 100 | 83 |
| 60°C | 2·5 | 100 | 86 | 79 | 100 | 100 | 100 | 100 | 95 |
| | 7.0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | 9.5 | 100 | 86 | 49 | 29 | 100 | 83 | 67 | 67 |

Aqueous solutions of the platomycins (50μg/ml) were adjusted to pH 2.5, 7.0 and 9.5 and kept at 30°C and 60°C. After storage for the specified hours, an aliquot of each solution was adjusted to pH 7.0 and measured for activity against *B. subtilis* KY 4273.

## Chromatographic Comparison of the Platomycins

Rf values on paper and silica gel thin-layer chromatograms (TLC) of platomycins A and B are shown in Tables 2 and 3, respectively. A mixture of platomycin A, platomycin B, bleomycin $A_2$, $A_5$, $B_2$ and $B_4$ was dissolved in a small amount of 0.05 M aqueous ammonium formate and chromatographed over a CM-Sephadex C-25 column (1.2×50 cm). The column was washed with 50 ml of 0.05 M aqueous ammonium formate and then eluted gradiently (0.05 M~1.0 M) with aqueous ammonium formate. As shown in Fig. 4, five peaks were observed by UV monitor at 293 nm. All peaks have antibacterial activity. Each peak was collected and the solutions desalted with Amberlite IRC-50($H^+$) and Sephadex LH-20. The antibiotics in each fractions were identified by TLC. The first peak (0.35 M) contained bleomycin $A_2$, the second $\frac{2}{3}$(0.45 M) bleomycin $B_2$, the third (0.60 M) bleomycin $A_5$, the fourth (0.68 M) a mixture of platomycin A and bleomycin $B_4$ and the last peak (0.83 M) platomycin B.

Table 2. Rf values of the platomycins on paper chromatography

| Solvent system | Rf value | |
|---|---|---|
| | Platomycin A | Platomycin B |
| 10% Ammonium chloride | 0.67 | 0.63 |
| 5%-Ammonium chloride | 0.65 | 0.60 |
| Water saturated *n*-butyl alcohol | 0.00 | 0.00 |
| *n*-Butyl alcohol-acetic acid-water (3:1:1) | 0.02 | 0.01 |
| Water-saturated *n*-butyl alcohol containing 2% *p*-toluenesulfonic acid 2% piperidine | 0.02 | 0.01 |
| Water-saturated ethylacetate | 0.00 | 0.00 |

Antibiotics were detected by bioautography on agar trays seeded with *B. subtilis* KY 4273.

## Acid Hydrolysis of the Platomycins*

Platomycin A, phleomycin and bleomycin were hydrolyzed by 6 N HCl at 105°C for 24

* Platomycins and phleomycin were Cu-complexes, and bleomycin Cu-free commercial material.

hours. The hydrolysates were compared by paper chromatogaphy (Fig. 5) and paper electrophoresis (Fig. 6) as described by TAKITA *et al.*[3] Results indicated that platomycin A might contain most of the components of bleomycin and phleomycin with the exception of 2′-(2-aminoethyl)-2, 4′-bithiazole-4-carboxylic acid (**VI**, Figs. 5 and 8), present in the bleomycin hydrolysate and β-alanine, present in the phleomycin hydrolysate. Platomycin A and bleomycin were also decomposed by methanolysis with Dowex 50×4 as described by TAKITA *et al.*[4] After removing the resin, the hydrolysate was concentrated to dryness and acetylated using pyridine and acetic anhydride. The sugar components in the hydrolysates of both antibiotics and their acetylated derivatives were compared by TLC (Fig. 7). Two sugar components were present in the methanolysis products of both platomycin A and bleomycin, with identical Rf values.

The acid hydrolysate and the methanolysis products of platomycin B were also examined. Results showed that the components of platomycin B are very similar to those of platomycin A. The determination of structure of platomycins will be the subject of subsequent studies.

Table 3. Rf values of the platomycins and related compounds on silica gel TLC.

| | Solvent system | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Platomycin A | 0.43 | 0.73 | 0.62 | 0.15 |
| Platomycin B | 0.10 | 0.60 | 0.47 | 0.05 |
| Victomycin | 0.26 | 0.78 | 0.40 | 0.45 |
| Bleomycin $A_2$ | 0.27 | 0.45 | 0.52 | 0.03 |
| Bleomycin $A_5$ | 0.05 | 0.60 | 0.28 | 0.05 |
| Bleomycin $B_2$ | 0.45 | 0.80 | 0.88 | 0.03 |
| Bleomycin $B_4$ | 0.10 | 0.75 | 0.68 | 0.03 |
| Zorbonomycin B | 0.33 | 0.76 | 0.84 | 0.19 |

Solvent 1 Chloroform-methanol-17% ammonia water (2:1:1 upper layer)
2 10% Ammonium acetate-methanol (1:1)
3 Methanol-10% ammonium acetate-10% ammonia water (10:9:1)
4 0.05 M Citrate buffer (pH 6.9)
Antibiotics were detected by bioautography on agar trays seeded with *B. subtilis* KY 4273

Fig. 4. CM Sephadex chromatography of platomycins and related compounds.
Column: CM Sephadex C-25 (1.2×50 cm)
Elution: 0.05~1.0 M ammonium formate
Flow rate: 40 ml/hour

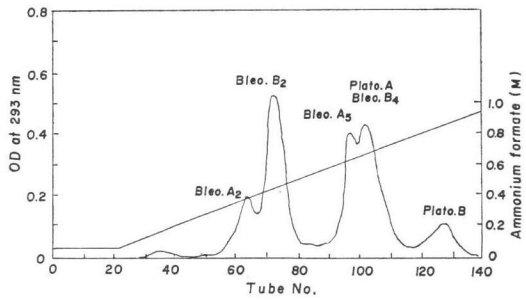



Fig. 5. Paper chromatography of acid hydrolysate of platomycin, bleomycin and phleomycin.
Solvent system: *n*-butyl alcohol-acetic acid-water (4:1:2)
Coloration: ninhydrin spray (p: purple, g: green, y: yellow, b: brown)

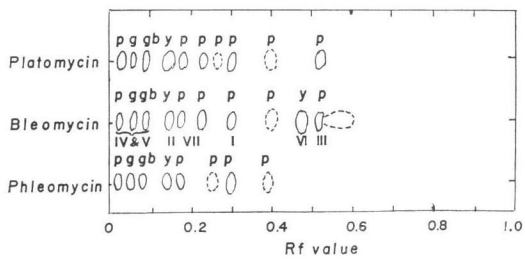



Fig. 6. Paper electrophoresis of acid hydrolysate of platomycin and bleomycin
Buffer solution: formic acid-acetic acid-water (25:75:900, pH 1.7)
Condition: 3,000 volts, 30 min.
Coloration: ninhydrin spray (p: purple, y: yellow, b: brown)
Rm value of alanine =1

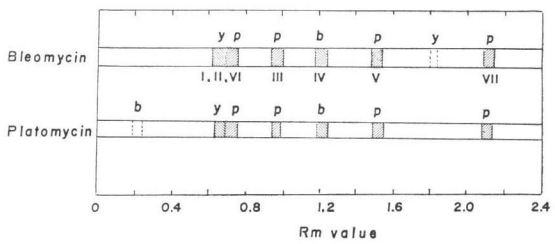


Fig. 7. Silica gel TLC of the methanolysis products of platomycin and bleomycin
A. Sample: methanolysis product
Solvent system; *n*-propyl alcohol-ammonia-water (3:1:1)
Coloration: anisaldehyde
B. Sample: acetylated derivatives of the methanolysis product
Solvent system: ethyl acetate-chloroform (9:1)
Coloration: anisaldehyde

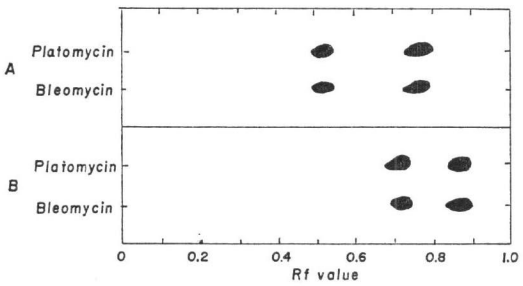
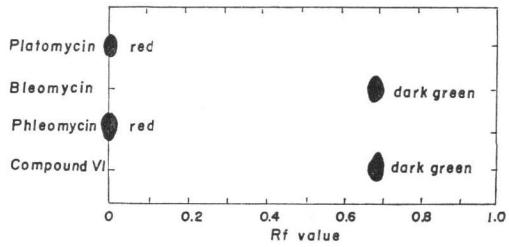



Fig. 8. Identification of compound VI on Silica gel TLC containing fluorescence
Solvent system: *n*-propyl alcohol-ammonia-water (3:1:1)
Coloration: UV irradiation at 254 nm
Compound VI was extracted from PPC of the acid hydrolysate of bleomycin




## Discussion

The physicochemical properties described above and the biological properties presented in the previous paper[1] indicated that platomycins A and B are related to but different from the phleomycin-bleomycin group of antibiotics. Since the ratio of the intensity of the absorption at 244 to that at 293 nm of platomycin A is 1.31 and of platomycin B is 1.23, both antibiotics are similar to the bleomycins,[5] zorbonomycin B[6] and victomycin[2] (ratio of 1.1~1.4) and different from phleomycin,[7] zorbamycin,[6] zorbonomycin C,[6] YA-56X[8] and YA-56Y[8] (ratio of 2.7~3.0). Bleomycins A ($A_1$, demethyl $A_2$, $A_2$, $A_2$'-a, $A_2$'-b, $A_4$, $A_5$ and $A_6$) and bleomycin $B_1$ give negative SAKAGUCHI reactions, while bleomycins B ($B_2$, $B_3$ $B_4$, $B_5$ and $B_6$) give positive SAKAGUCHI reactions.[9,10,11] Platomycins A and B give positive SAKAGUCHI reactions and therefore are related to the bleomycin B group. CM-Sephadex chromatography (Fig. 4) did not separate platomycin A from bleomycin $B_4$. Victomycin is also known to be eluted with bleomycin $B_4$.[2] Platomycin A was differentiated from both victomycin and bleomycin $B_4$ by TLC. The Rf values of platomycin A, victomycin and bleomycin $B_4$ were 0.43, 0.26 and 0.10 with solvent 1, 0.62, 0.40 and 0.68 with solvent 3, and 0.15, 0.45 and 0.03 with solvent 4, respectively (Table 3). The platomycins were also differentiated from zorbonomycin B on TLC (Table 3). Furthermore platomycin A is different from bleomycins $B_2$, $B_3$ and $B_6$, since all these components, on chromatography over CM-Sephadex, were eluted with ammonium formate concentrations different from that used for bleomycin $B_4$. Similarly, platomycin B is different from bleomycins $B_2$, $B_3$ and $B_4$. No differentiation of platomycin B from bleomycins $B_5$ and $B_6$ by chromatographic procedures is available at present. The hydrolysis data indicate the platomycins differ from the bleomycins in the bleomycinic acid moiety.

Acknowledgement

The authors are very grateful to Dr. A. C. SINCLAIR and his associates in Abbott Laboratories, North Chicago, Ill., U.S.A. for their kind advice and encouragement.

They are also very thankful to Dr. A. D. ARGOUDELIS, The Upjohn Company, for his kind supply of zorbamycin and zorbonomycin B and to Dr. T. OKUDA, Microbial Chemistry Reseach Laboratory, Tanabe Seiyaku Co., Ltd. for his kind supply of YA-56X.

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