CHEMICAL STUDIES ON BLASTMYCIN. III

GAS-LIQUID CHROMATOGRAPHY OF ANTIMYCIN A-BLASTMYCIN ANTIBIOTICS*

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The antimycin A-blastmycin antibiotics are strong fungicides which have been separated into the components antimycin A_1, A_2, A_3 (blastmycin) and A_4. The structures of A_1 and A_3 were elucidated as I. Gas-liquid chromatographic studies of the antibiotic derivatives revealed additional components. Degradation products from the antibiotics were also investigated by gas-liquid chromatography to determine individual antibiotic structures. The blastmycin complex and antimycin A complex can be classified respectively into four and about nine members, which differ in alkyl side chain and acyloxy groups as listed in Fig. 3.

Blastmycin[1] was found as a strong fungicide and was determined to be a member of antimycin A complex[2], which was separated into antimycin A_1, A_2, A_3 (blastmycin) and A_4.[3] Their chemical structures[4,5,6,7] were elucidated as I; antimycin A_1 R=*n*-C_6H_{13}-, R'=$(CH_3)_2CHCH_2$- and antimycin A_3 (blastmycin) R=*n*-C_4H_9-, R'=$(CH_3)_2CHCH_2$-.

Fig. 1. Gas-chromatogram of TMS-blastmycin.

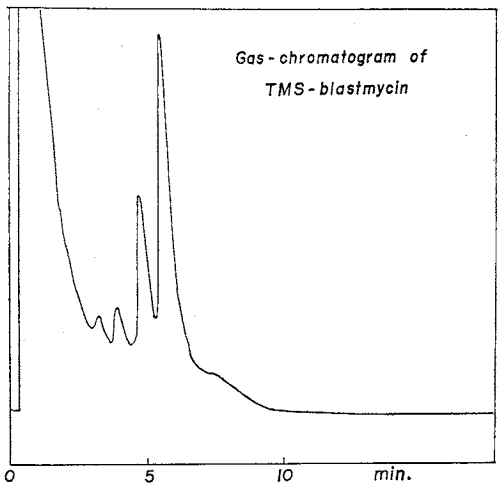
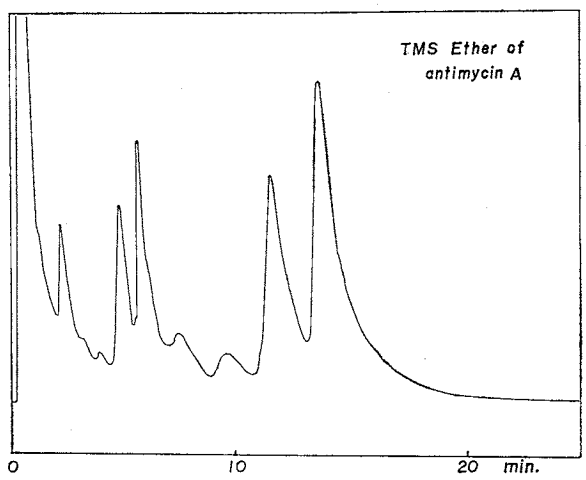
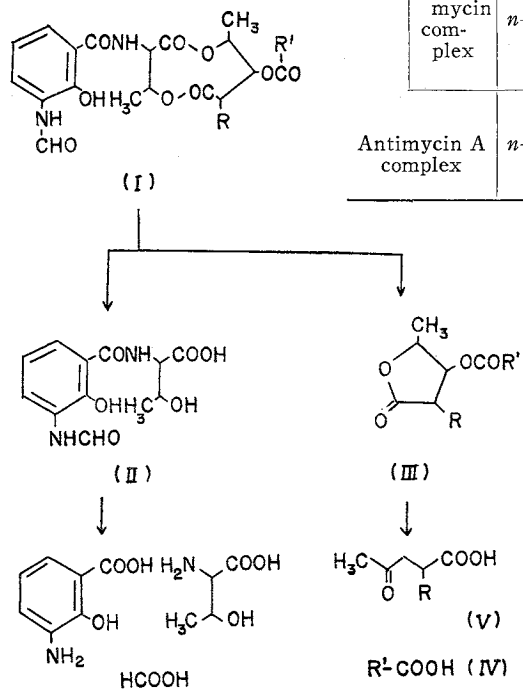


Fig. 2. Gas-chromatogram of TMS-antimycin A.



* This part of study was reported in 11th Symposium on the Chemistry of Natural Product, Oct. 23rd 1967, Kyoto, Japan[8]. Authors propose to express these antibiotics as Antimycin A-blastmycin antibiotics, including unclarified antibiotics virosin, antipiriculin, antifungal substance 720A and phyllomycin *etc.*

Fig. 3. Degradation scheme of the antibiotics.



	Alkyl side chains		Component
	R	R'	
Blastmycin complex	n-C_4H_9-	$(CH_3)_2CH$- n-C_3H_7- $CH_3CH_2CH(CH_3)$- $(CH_3)_2CHCH_2$-	Antimycin A_3 Blastmycin
Antimycin A complex	n-C_6H_{13}-	$(CH_3)_2CH$- $CH_3CH_2CH(CH_3)$- $(CH_3)_2CHCH_2$-	Antimycin A_1

Recently trimethylsilyl ether or trifluoroacetate derivatives of the antibiotics was prepared and submitted to gas-liquid chromatography. On silicone oil column (1.5 % SE 30) TMS-blastmycin, which was considered to be a single component, gave four peaks and TMS-antimycin A gave about nine peaks as shown in Figs. 1 and 2.*

Mild alkaline treatment of blastmycin complex and antimycin A complex afforded in high yield two degradative components, blastmycic acid (II) and blastmycinone or antimycinone (III), of which gas chromatograms are shown in Figs. 4 and 5. Under these conditions (20 % DEGS column), blastmycinone gave 2 peaks and 2 little signals, and antimycinone gave about nine peaks with broad peaks at

Fig. 4. Gas-chromatogram of blastmycinone.

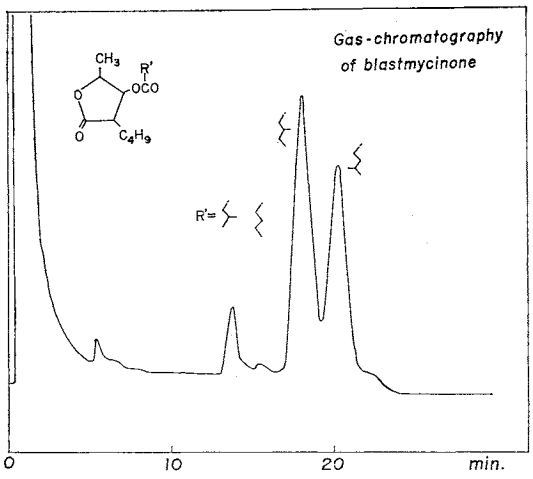
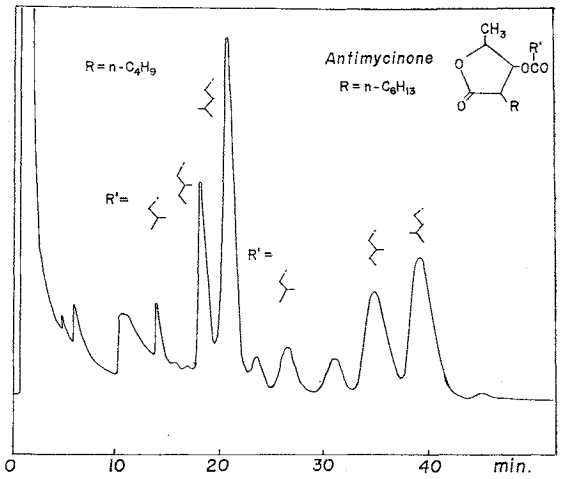


Fig. 5. Gas-chromatogram of antimycinone.



* These signals must have originated from the derivatives, for the antibiotics are not so unstable in these conditions, and some degradation products were not well separated on these less-polar columns.

Fig. 6. Gas-chromatogram of volatile acid from blastmycin complex.

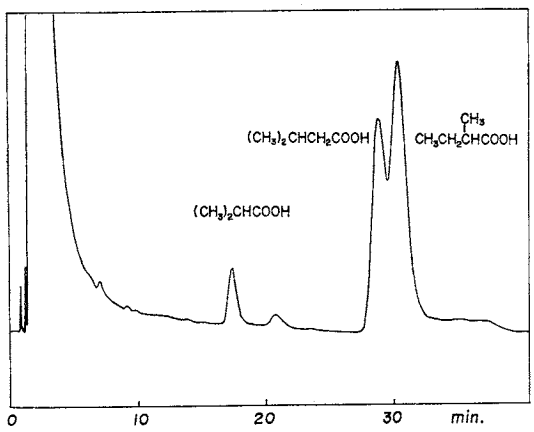
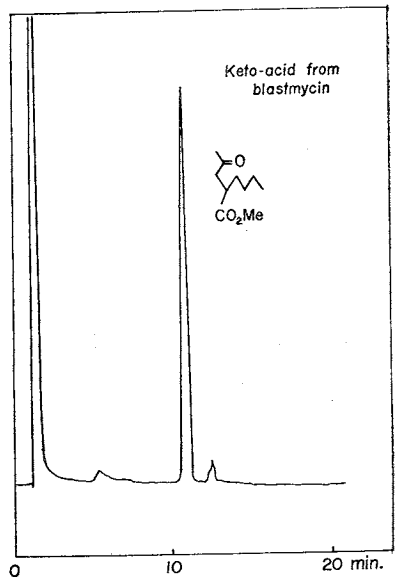


Fig. 7. Gas-chromatogram of keto-acid from blastmycin complex.



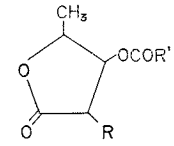
lower retention times. They were similar to the patterns of the TMS-antibiotics. Blastmycic acid, an amino acid moiety of the antibiotics, was degraded to 3-amino-salycilic acid, L-threonine and formic acid and did not yield other related components. Thus the structural variety of the original antibiotics must reside in the alkyl side chain or the acyloxy group of the γ-lactones (III).

By vigorous boiling in alkaline solution, III was degraded to volatile acids (IV) and ketonic acids* (V). The analysis of these moieties, having R and R′ side chain respectively, permits the structural elucidation of γ-lactones (III) and original antibiotics (I).

Blastmycinone (III), by alkaline treatment, gave four volatile acids; small amount of iso-butyric acid, *n*-butyric acid, major amount of iso-valeric acid and 2-methyl-butyric acid (Fig. 6), in order of increasing retention times. But from the ketonic acid portion, only one peak was detected after methylation with diazomethane (Fig. 7), and it was found to be methyl 2-*n*-butyllevulinate** (m/e 186 (M^+), 171, 155, 143, 129, 127). The structural assignment of peaks in Fig. 4 was carried out through recombination of these moieties or direct separation and degradation of every peak. So blastmycinone and blastmycin were concluded to be a mixture

Table 1

Antimycinone



Tr	R′	R
5.1	—	—
6.0	—	—
11.0	—	—
14.1	$(CH_3)_2CH-$	*n*-C_4H_9-
15.6	minor	
16.8	minor	
18.3	$CH_3CH_2CH(CH_3)-$	*n*-C_4H_9-
20.5	$(CH_3)_2CHCH_2-$	*n*-C_4H_9-
23.2	minor	
26.2	$(CH_3)_2CH-$	*n*-C_6H_{13}-
30.7	complex	
34.6	$CH_3CH_2CH(CH_3)-$	*n*-C_6H_{13}-
38.7	$(CH_3)_2CHCH_2-$	*n*-C_6H_{13}-
45.0	minor	

* Van Tamelen *et al.* (J. Am. Chem. Soc. 81 : 750, 1959) obtained levulinic acid by alkaline treatment of synthetic 3-benzoxy-velerolactone, similarily 2-alkyllevulinic acid was formed from III.

** Yonehara *et al.*[3] reported *n*-butylmalonic acid formation from blastmycin and Uzu *et al.*[7] obtained *n*-hexylmalonic acid from antimycin A_1 as R-side chain containing moieties.

Fig. 8. Gas-chromatogram of volatile acids from antimycinone.

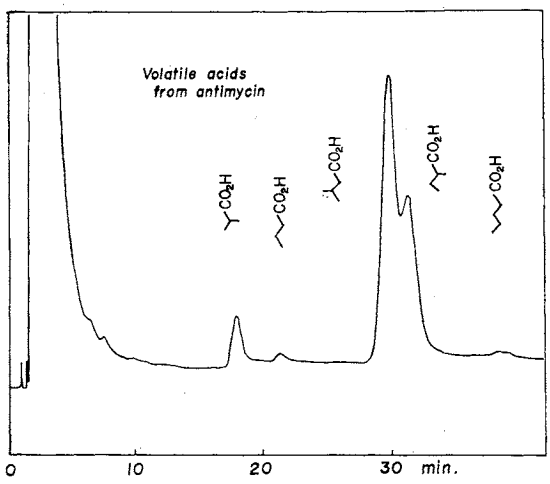
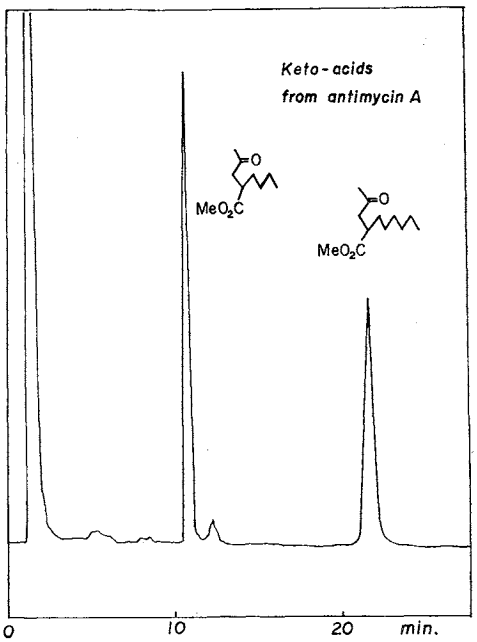


Fig. 9. Gas-chromatogram of keto-acids from antimycinone.



of R′=$(CH_3)_2CH$-, n-C_3H_7-, $CH_3CH_2CH(CH_3)$- and $(CH_3)_2CHCH_2$- groups with R=n-C_4H_9- in III and I.

Antimycinone, through the same treatment, afforded *iso*-butyric acid, n-butyric acid, isovaleric acid, 2-methyl-butyric acid, n-valeric acid and n-caproic acid as volatile acids (IV) (Fig. 8). As ketonic acids (V), two peaks were detected after methylation and they were found to be methyl 2-n-butyllevulinate and methyl 2-n-hexyllevulinate (m/e 214 (M^+), 199, 183, 171, 155 and 129, Fig. 9). The combination of these moieties, *i. e.* of the side chains in antimycinone, were determined through preparative gas-chromatography of antimycinone (Fig. 5) and degradation of each peak. Table 1 shows the combination of R and R′ side chains on the γ-lactone skeleton, and consequently analogous members of antimycin A and blastmycin antibiotics should be described in the same manner.

Conclusively, the antimycin A complex is as depicted in I with R, R′ side-chain combinations as shown in Fig. 3. The relationship between the structures and classification A_1~A_4 are not yet clarified. But considering the difficulty of separating isovaleric acid and 2-methylbutyric acid, comixtures with such acyl groups would not be distinguished easily, and would be considered single antibiotic.

So authors wish to designate again antimycin A_1 as the antibiotic containing R=n-C_6H_{13}- and R′=$(CH_3)_2CHCH_2$- of I, and antimycin A_3 (blastmycin) as R=n-C_4H_9- and R′=$(CH_3)_2CHCH_2$-.

Experimental

Trimethylsilyl ether derivative. Antimycin A (mp 146.5°C, 5 mg) or blastmycin (mp 167°C, 5 mg) was dissolved in THF (0.5 ml) and heated for 5 minutes with hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.1 ml). After removal of most reagent in an air stream, TMS-ethers were chromatographed on a silicone oil column: 1.5 % SE-30 on diatomaceous earth, Shimalite (Shimadzu Seisakusho Ltd.) 80~100 mesh, 1.5 m×3 mm i. d., He 57 ml/min., at 151°C.

Trifluoroacetate. Each antibiotic (5 mg) was reacted in THF (0.5 ml) with one drop of trifluoroacetic anhydride and pyridine at room temperature for 15 minutes. After removal of most reagent, trifluoroacetate in H_2O was extracted with benzene and dried over Na_2SO_4, then THF solution was analysed under the above conditions.

Hodrolysis.

i) Hydrolysis of the antibiotics on a microscale was carried out as follows: Several milligrams of antibiotic was treated at room temperature with 1 ml of 3 N sodium hydroxide till disappearance of the crystals. Separated colorless oil was extracted with petroleum ether (bp 35~44°C) and dried over Na_2SO_4. After removal of the solvent, γ-lactones in acetone were analysed on gas-liquid chromatography column of diethylene-glycol succinate 20 % on Chromosorb W 60~80 mesh, 2 m×3 mm i. d., N_2 30 ml/min. at 160°C.

Analysis of water layer was carried out as in Ref. 4.

ii) Several milligrams of antimycinone or blastmycinone was hydrolysed with 3 N sodium hydroxide at 95°C for 1 hour. Ethereal extract of acidified reaction mixture contained lower alkyl acids and alkyllevulinic acid. The alkyl acids were analysed on Octoil S-Behenic acid (1 : 1) 25 % on Celite 545-SK 60~80 mesh, 4 m×3 mm i. d., N_2 30 ml/min. at 150°C. Keto acids were methylated with diazomethane then chromatographed on 20 % DEGS, 2 m×3 mm i. d., N_2 30 ml/min. at 140°C.

Acknowledgement

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