INCORPORATION OF L-METHIONINE-METHYL-^{14}C INTO GENTAMICINS. III. CHROMATOGRAPHIC SEPARATION AND DEGRADATION OF COMPONENTS OF METHYL-^{14}C-GENTAMICIN COMPLEX

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A selective and high incorporation of radioactivity from methyl-^{14}C-L-methionine into gentamicin components was shown by LEE and his co-workers[1]. Methyl-^{14}C-gentamicin complex was prepared, fermentatively, by employing *Micromonospora purpurea*, and the resulting radioactive antibiotics isolated and purified[2]. The results on a chromatographic separation of components from the radioactive gentamicin complex[3], acid hydrolysis of each component, and determination of radioactivity incorporated into each component and subunit are reported in this paper.

Materials and Methods

Adsorbents. Amberlite IRA-401S resin and silica gel powder (60~300 mesh) were purchased respectively, from Malinckrodt Chemical Co. and J. T. Baker Chemical Co.

Column chromatography. Redioactive gentamicin complex sulfate (2.5 g) was converted to base by means of Amberlite IRA 401S (200 g in OH^- from) column chromatography. Gentamicin complex base (843 mg) was packed on a silica gel (150 g) column (2.8× 96.2 cm), and components were separated and isolated by eluting the column with the lower phase of chloroform-methanol-17% ammonia (2:1:1 by volume), at a flow rate of 10 ml/20 min.

Hydrolysis. Gentamicin components, at concentrations of 10 mg each/ml 6 N HCl, were hydrolyzed in sealed tubes for 2 hours in a boiling water bath.

Paper chromatography. Paper chromatograms of the gentamicin components were developed in a descending system of the lower phase of chloroform-methanol-17% ammonia (2:1:1 by volume), and hydrolysates of the respective components were separated in an ascending system of *n*-propanol-pyridine-acetic acid-water (25:10:3:12 by volume).

Assay of radioactivity. A Nuclear Chicago scanner (Model 1002) and an Intertechnique

Fig. 1. Radioactivity scan of gentamicin C_1, C_2 and C_{1a} components

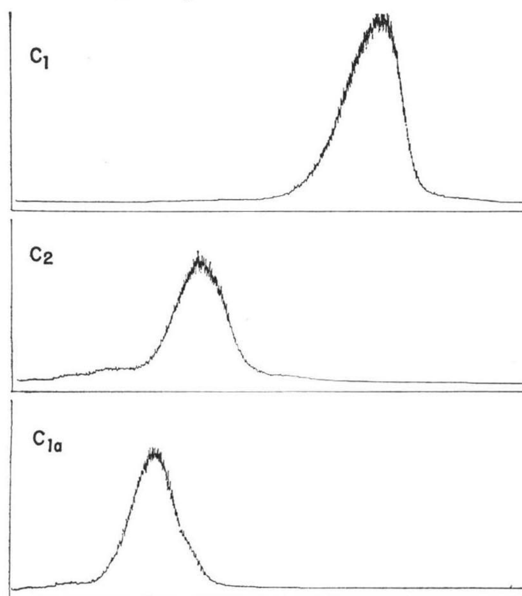
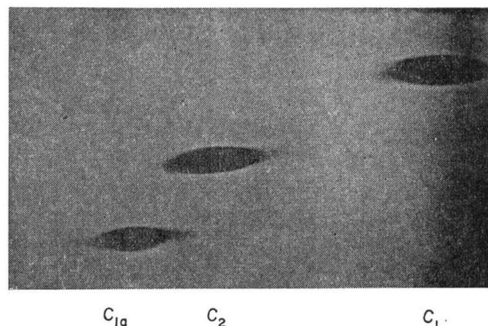


Table 1. Conversion of gentamicin sulfate to base

	Weight (mg)	Bio-activity (μg/mg)	Radio-activity (μCi)	Specific radio-activity (μCi/mg)
Sulfate	2,508	476	1,534	0.612
Base	843	868	1,138	1.348

Fig. 2. Bioautograms of gentamicin C_1, C_2 and C_{1a} components



scintillation spectrometer (Model SL 30) were used for the scan and measurement of radioactivity.

Results

When radioactive gentamicin complex sulfate was converted to base by using an IRA 401S column, specific radioactivity and bioactivity were changed from 0.612 μCi/mg and 476 μg/mg sulfate, respectivity, to 1.349 μCi/mg and 868 μg/mg base (Table 1).

From an input of 843 mg of the radioactive gentamicin complex base, 296 mg of the C_1 component, 34 mg of the C_2 component and 113 mg of the C_{1a} component were separated and isolated chromatographically (Table 2). The specific radioactivity of the isolated C_1, C_2 and C_{1a} components were 1.537 μCi, 1.099 μCi and 0.919 μCi/mg base respectively (Table 2). Radiochemical and biological properties of the components were quite satisfactory as shown in Fig. 1 and Fig. 2.

Radioactivity distribution of 2-deoxystreptamine-C_1-purpurosamine-garosamine was 0 : 226 : 227; that of 2-deoxystreptamine-C_2-purpurosamine-garosamine was 0 : 16.4 : 18.5, and that of 2-deoxystreptamine-C_{1a}-purpurosamine - an unknown substance (which migrated between C_{1a}-purpurosamine and

Fig. 3. Radioactivity scan of ninhydrin-treated gentamicin C_1 hydrolysate.

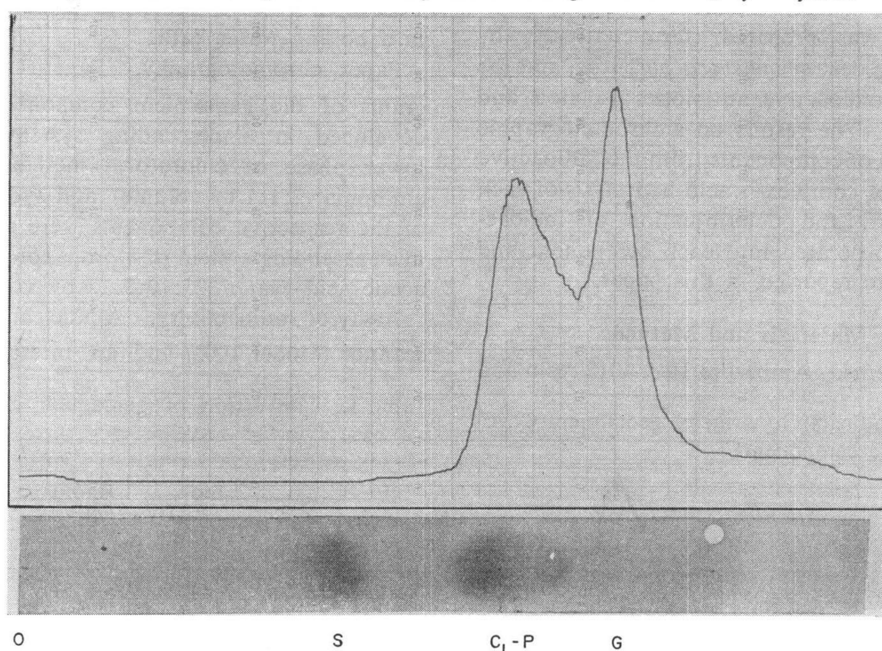


Table 2. Separation of components of gentamicin complex base.

Fraction	Tube No.	Component	Weight (mg)	Bioactivity (μg/mg)	Radio-activity (μCi)	Specific redioactivity (μCi/mg)
1	276～342	C_1	296.0	690	455.0	1.537
2	343～427	C_1+C_2	320.5	907	383.6	1.197
3	428～454	C_2	34.2	802	37.6	1.099
4	455～474	C_2+C_{1a}	4.6	150	3.8	0.816
5	475～582	C_{1a}	112.7	642	103.5	0.919
6	583～709	C_{1a}+polar	18.4	426	15.9	0.865
7	710～778	polar	10.5	551	—*	—

* —: Not determined.

Table 3. Hydrolysis of gentamicin componente.

Component hydrolyzed			Radioactivity of subunit released (μCi)			2-Deoxy-streptamine	Garosa-mine
Component	Weight (mg)	Radioactivity (μCi)	Polar	Unknown	Purpurosamine		
C_1	3.9	5.994	0	0	225.95 (C_1-purp)	0	226.73
C_2	2.7	2.967	2.63	0	16.43 (C_2-purp)	0	18.54
C_{1a}	3.2	2.941	2.65	34.33	0 (C_{1a}-purp)	0	66.52

Fig. 4. Radioactivity scan of ninhydrin-treated gentamicin C_2 hydrolysate.

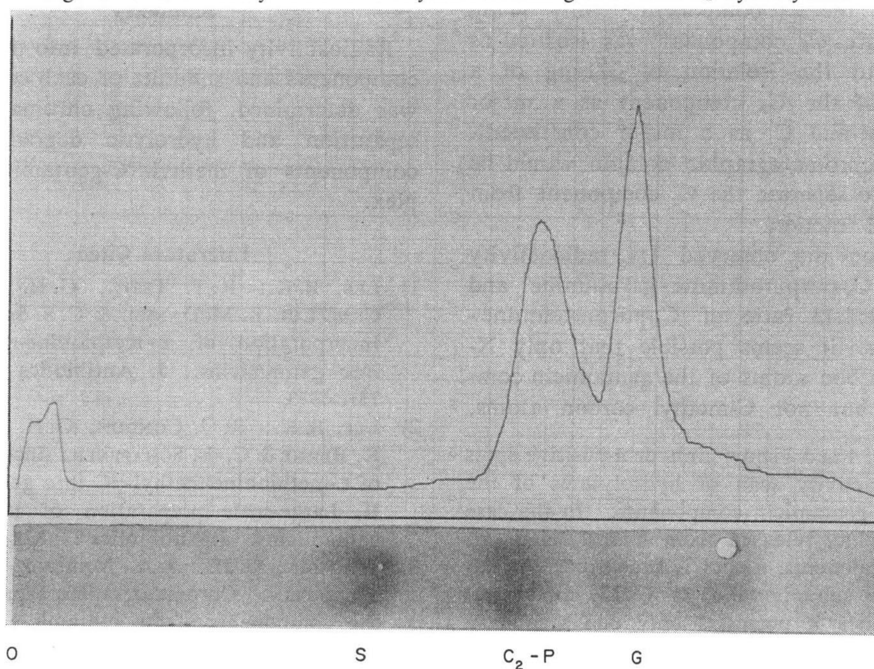
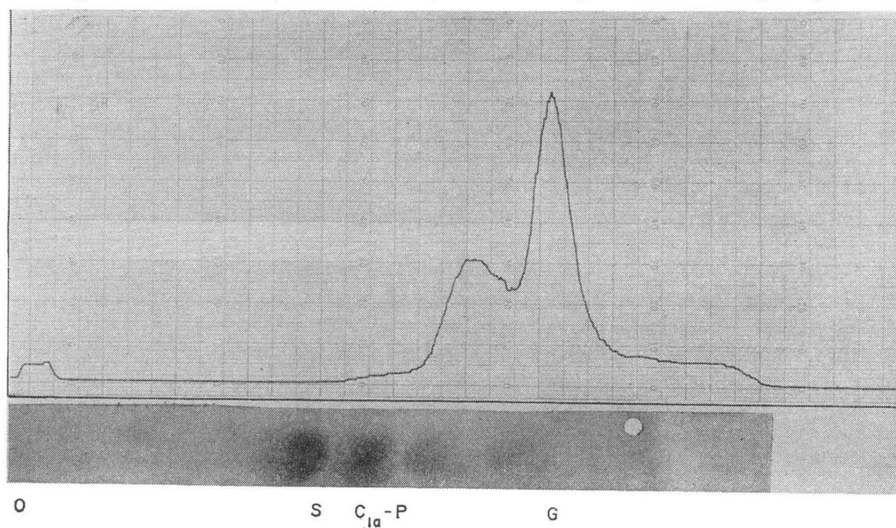


Fig. 5. Radioactivity scan of ninhydrin-treated gentamicin C_{1a} hydrolysate.



garosamine on papergrams) - garosamine was 0:0:34.3:66.5 (Table 5). Trace amounts of radioactivity were also detected in the origin of papergrams from the hydrolysates of C_2 and C_{1a} components (Table 3, Figs. 3, 4 and 5*).

Discussion

As shown in the results, great difficulty was encountered in chromatographically separating the gentamicin C_2 component free from the C_1 component. Only 34 mg of the pure C_2 component was isolated as opposed to the isolation of 321 mg of a mixture of the C_2 component as a major constituent and C_1 as a minor constituent. Another chromatographic column would be required to separate the C_2 component from the mixed fraction.

Based on the observed 1:1 radioactivity ratio of C_1-purpurosamine - garosamine and on the 1:1.13 ratio of C_2-purpurosamine - garosamine, it seems possible that only N-methyl carbon atoms of the gentamicin components, but not C-methyl carbon atoms, are derived from methionine. The presence of an unknown substance, which migrates between C_{1a}-purpurosamine and garosamine on the radioautograms, in the hydrolysate of C_{1a} component is unexplainable. Incorporation of radioactivity into the unknown substance amounts to about half of that incorporated into the garosamine moiety. Further studies are in progress to resolve the origin of the methyl groups.

* Figs. 3, 4 and 5 show ninhydrin-positive spots and radioactivity scan of hydrolysates of the respective gentamicin components. In the case of garosamine, released from hydrolysis of all of the components, a spot is shown corresponding to the subunit which is weakly ninhydrin-positive since it possesses only one secondary amine group.

Summary

Radioactivity incorporated into individual components and subunits of each component was determined, following chromatographic separation and hydrolytic degradation of components of methyl-^{14}C-gentamicin complex.

Literature Cited

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3) WAGMAN, G. H.; J. A. MARQUEZ & M. J. WEINSTEIN: Chromatographic separation of the components of the gentamicin complex. J. Chromatogr. 34: 210~215, 1968