Tunicamycin (I) is a nucleoside antibiotic complex, produced by *Streptomyces lysosuperficus*.1) In the preceding communication,2) the structure of its degradation product, tunicaminyl uracil (II), was reported. This paper deals with the isolation of other degradation products and the gross structure of tunicamycin.

Tunicamycin was hydrolyzed with 3 N hydrochloric acid at reflux for 3 hr and the hydrolyzate was extracted with ethyl ether. Concentration of the ethyl ether extract gave the mixture of fatty acids (III). IR \( \nu_{\text{max}} \) cm\(^{-1}\): 1650, 1700 (\(-\text{CH} - \text{CH} - \text{COOH}\)), 1310, 980 (trans \(-\text{CH} - \text{CH}\)), 1365, 1380 (isopropyl). NMR (in CDCl\(_3\)) \( \delta \): 5.73 (1H, doublet, \( J=16 \), trans \(-\text{CH} - \text{CO}\)), 6.98 (1H, sextet, \(-\text{CH}\)), 1.27 (16–18 H, singlet, \(-(\text{CH}_2)_n-\)), 0.88 (doublet, two CH\(_3\) of isopropyl).

Thus tunicamycin was shown to be not a single compound, but a mixture of homologous antibiotics, each of which contains one mole of fatty acid with different carbon chain length. The IR and NMR spectra of the ether fraction indicated that the major components of fatty acids fraction were trans \( \alpha,\beta \)-unsaturated iso acids. These were converted into the methyl esters and analyzed by gas-liquid chromatography (1 m column containing Silicone GE SE–30) and GLC-mass spectrometry. The gas-liquid chromatogram of methyl esters of III showed four major peaks and seven minor peaks. The mass spectrum (GC–MS) of the methyl ester of acid (III A), which gave the most intense peak in the gas-liquid chromatogram, showed M\(^+\) ion peak at \textit{m}/\textit{e} 254 and the fragmentation peaks at M–31, M–32 and the peak at \textit{m}/\textit{e} 113, which is characteristic to the spectrum of \( \alpha,\beta \)-unsaturated acid.3) The same fragmentation pattern was observed in the mass spectra of three methyl esters, of which molecular weights were 240, 268 and 282.
When III was hydrogenated with platinum black catalyst, it absorbed about 1 mole of hydrogen and gave the mixture (IV) of corresponding saturated acids, which were then esterified and analyzed as before. The mass spectra (GC-MS) of four major methyl esters indicated that their molecular weights were 242, 256, 270 and 284. Their terminal branching (iso) structure was confirmed by their mass spectra which showed intense peaks at M–C₃H₇ and the peaks corresponding to the similar fragmentations to that of 10-methylundecanoic acid methyl ester. Oxidation of III with potassium permanganate afforded the mixture of acids, which was then esterified. The gas-liquid chromatogram showed four major peaks corresponding to C₁₂, C₁₃, C₁₄ and C₁₅ iso acids.

To confirm the presence of double bond in fatty acid residue of tunicamycin itself, tunicamycin was hydrogenated with platinum oxide catalyst. Hydrolysis of the reduced tunicamycin yielded the saturated fatty acids (IV). These results indicated that the main fatty acids* in tunicamycin complex were α,β-unsaturated iso acids, i.e. 13-methyl-2-tetradeconoic acid (III A), 14-methyl-2-pentadecenoic acid (III B), 12-methyl-2-tridecenoic acid (III C) and 15-methyl-2-hexadecenoic acid (III D). We propose the names tunicamycin A, B, C and D for tunicamycins, which contain the iso acid III A (C₁₅), III B (C₁₆), III C (C₁₄) and III D (C₁₇), respectively.

After removal of fatty acid fraction and insoluble matters in water, the hydrolysate of tunicamycin was passed through Dowex 50 W resin, which was then eluted with 0.3 N hydrochloric acid. The eluate was concentrated to give a crystalline hydrochloride, which was identified as D-glucosamine hydrochloride by paper chromatography and IR and NMR spectra.

When tunicamycin was hydrolyzed with 3 N hydrochloric acid for 1 hr at 100°C, the precipitate (fraction P) separated. This compound was purified by chromatography on silica gel. mp 228–230°C (dec.), UV λ_max nm (E₁%1cm) 259 (170). The IR spectrum showed the presence of a peptide bond in fraction P. Hydrolysis of fraction P gave tunicaminy uracil (II) and fatty acid (III). This fact indicated that the amino group of tunicamine residue is substituted by fatty acid.

The ¹H NMR of tunicamycin complex (in pyridine-d₅) showed a signal at δ 2.22, which was assigned to the acetamido group of N-acetyl glucosamine residue. Tunicaminy uracil (II) and fraction P showed reducing activity, while tunicamycin was non-reducing. This fact suggested that C–11' of tunicamine residue was linked to the anomeric carbon of glucosamine with a glycosidic linkage. The specific rotation of tunicamycin complex was [α]_D^20 +61.6° (c=0.58, pyridine), while that of fraction P was [α]_D^20 +12.6° (c=0.45, pyridine). If we assume that the molecular weight of tunicamycin is 830.9 and that of fraction P is 627.7, their molecular rotations are +512×10⁵ and +79×10⁶, respectively. From the high molecular rotation of tunicamycin relative to the corresponding value for fraction P and methyl N-acetyl-α-D-glucosaminide ([M] = +247×10⁶ in H₂O), the α-α-linkage⁶) between C–11' of tunicamine and C–1” of N-acetyl glucosamine was suggested. The IR spectrum of tunicamycin also suggested α-α-linkage, because no peak was observed around 890 cm⁻¹.⁷)

The permethylated derivative (V) of tunicamycin complex was prepared by the method described by Hakomori.⁸) In the mass spectrum (sample temp. 240°C), V showed peaks at m/e 970, 984, 998 and 1012, which might be assigned to M⁺ ion peaks of permethylated tunicamycin C, A, B and D, respectively. Since these peaks differed from one another by 14 mass units, M⁺ peaks seemed to be overlapped with the fragmentation peaks of tunicamycin D, B or A. The relatively high peaks at m/e 694, 708, 722 and 736 might originate from the fragments which formed by the cleavage of N-acetyl glucosamine from

* The structures of other components of fatty acid fraction will be discussed in a later paper. One of the acids was shown to be iso-pentadecanoic acid by GC-MS analysis.
tunicamycins. The four peaks at m/e 308, 322, 336 and 350 could be accounted for by the fragmentations as indicated in structure V. We now assign the structure I to the main components of tunicamycin complex.

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