STUDIES ON TRICHOMYCIN. VI

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Recently a tentative structure of pimaricin$^{1}$ and partial structures of fungichromin,$^{2}$
lagosin,$^{3}$ and filipin$^{4}$ have been proposed, but few structural studies with regard to
members of hexaene and heptaene groups have been hitherto reported. It is the purpose
of the present paper to report structural studies on trichomycin A.

I. Polyenic Structure

It was already mentioned by Oroshnik et al.$^{5}$ that trichomycin belonged to a heptaene
group in polyene antibiotics. However there is no chemical evidence as to existence and
properties of groupings attached at the conjugate system.

Reduction of trichomycin A (I) with sodium borohydride gave an amphoteric substance
(II) which was purified by counter-current distribution. The ultraviolet spectrum of II
was nearly identical to the spectrum of I in the longer wavelength region. (Absorption
maxima of II occur at 364, 384.5 and 409 μm in the upper phase of the solvent system
used in counter-current distribution. Those of I occur at 363, 384.5 and 407 μm in the
same solvent system.) Reduction of I with lithium aluminum hydride also gave a similar
result showing that no structural change had taken place in the polyenic system.

Complete acetylation of I gave amorphous III which showed hydroxyl band in the
infrared. The ultraviolet spectrum of III was also nearly identical with the spectrum
of I, showing no hydroxyl or amino group had participated in the conjugate system.
(Absorption maxima of III occur at 363, 384.5 and 408 μm in the above solvent.) (Figs.
1 and 2).

Absence of allylic hydroxyl group was suggested by negative results of manganese
dioxide oxidation.

Oxidation of I with ozone gave gly­acetylated trichomycin A (III)
in methanol), which was identified as 2, 4-dinitro­
phenylhydrazone, and with chromium tri­
oxidation, and with chromium tri­
oxide gave 2, 4, 6, 8-decatetraene-1, 10-dial,
of which structure was estimated only by
infrared and ultraviolet because of its
 poor yield.

Detection of pentaenedial failed un­
fortunately, but because only one kind of
 tetraenedial except lower polyenic com­
 pounds was detected spectrally in the
 whole reaction mixture, it was confirmed
 that no substituent existed in the conjugate

![Ultraviolet absorption spectrum of acetylated trichomycin A (III) (in methanol)](image-url)
system unless the substituent was so large that the polyenic oxidation product could not be extracted under the used conditions.

When trichomycin A was subjected to a treatment with sodium hydroxide solution, extracted with ether, and chromatographed on alumina, the visible spectrum of the ether eluate with a single peak occurred at 418 mµ. The product so obtained, upon reduction with sodium borohydride, exhibited maxima at 374, 394 and 418 mµ, showing that the original chromophore included one carbonyl group and was reduced to a conjugate octaene system (Fig. 3). Furthermore, a conjugate nonaene system was found after reduction of less soluble fraction of the ether extract though only the highest peak was detected because of incomplete separation from octaene substance. These results indicate the

\[
\text{OHC-}\left(\text{CH=CH}\right)_5^- \\
\text{NaBH}_4 \\
\text{HOH}_2\text{C-}\left(\text{CH=CH}\right)_5^- \\
\text{OH}
\]

Fig. 3

(a) Ultraviolet spectrum of the ether extract obtained by alkaline treatment of I

(b) Ultraviolet spectrum of the alkaline degradation product treated with NaBH₄ in methanol
presence of partial structure (A) in trichomycin A.

II. Identification of Aminosugar

Upon hydrogenation with Adams' catalyst in glacial acetic acid, trichomycin A absorbed 10~11 mols of hydrogen to yield perhydro-trichomycin A (IV). Hydrolysis of IV gave the colorless and hygroscopic substance which showed positive reactions on tests with ninhydrin and triphenyltetrazolium chloride. Rf-values on paper partition chromatography using four solvent systems were in agreement with those of mycosamine.7 Although no isolable products could be obtained by acetolysis and methanolysis of IV as well as by hydrolysis, the products in all cases suggested the presence of aminosugar, probably mycosamine. The crystalline product was obtained by ethanolysis of N-dinitrophenyl derivative of IV. The analytical data showed that the product, chromatographed on alumina and recrystallized from benzene, was ethylglycoside of a basic moiety of the composition C_{12}H_{13}O_{4}N containing one mol of benzene as crystal-solvent. This product was identified, by infrared spectrum and mixed melting point, with ethyl N-dinitrophenyl mycosaminide (V) obtained by hydrogenation, dinitrophenylation and ethanolysis of pimaricin.

No consumption of periodic acid was observed in the oxidation of V at pH 4.5, while ethyl N-dinitrophenylglucosaminide consumed 2 mols of periodic acid, and N-dinitrophenylglucosamine 5 mols in the same conditions, showing that the cleavage of α-aminoalcohol system was not prevented by dinitrophenylation. These results indicated that the different behaviors of dinitrophenyl-amino groups were owing to whether the pyranose ring of aminosugars opened or not by the cleavage. If dinitrophenylamino group have been in cis position against at least one of neighboring hydroxyl groups, consumption of periodic acid would have occurred, and therefore it was concluded that the relationship between substituents at C_2, C_3 and C_4 on the ring of mycosamine, must be trans each other.

Mycosamine was detected by paper partition chromatography in the reduction product, indicating that hydrogenolysis also took place in the catalytic reduction.

Since there are only two conjugate systems, polyene group and p-aminophenyl group (see below), in trichomycin A with regard to double bond system, the occurrence of hydrogenolysis proved that mycosamine attached to either C_1 or C_16 in schema (A).

III. p-Aminobenzoyl Group

The presence of p-aminophenyl group in trichomycin A was already mentioned in the previous paper,6 and further studies have proved that alkaline treatments of I and II give p-aminoacetophenone and p-acetylaminoacetophenone respectively.

In addition, the original existence of p-aminobenzoyl group was suggested by the following results compared with absorption maxima of p-aminoacetophenone (316 mÅ),
p-acetylaminoacetophenone (287 mµ), p-acetylaminotoluidine (245 mµ) and p-aminotoluidine (236 mµ and 291 mµ).

1. When trichomycin A was hydrogenated absorbing 7 mols of hydrogen, the absorption maximum of the reduction product (VI) occurred at 320 mµ to 329 mµ (Fig. 4).

2. When trichomycin A was hydrogenated in glacial acetic acid containing acetic anhydride, the main product (VII) exhibited maximum at 286 mµ, and when the acetylated trichomycin A was oxidized with ozone. The main product (VIII) exhibited maximum at 287 mµ (Fig. 5).

3. By the reduction of VII or VIII with sodium borohydride, the maximum at 286-287 mµ disappeared and new maximum at 245 mµ appeared.

4. By the reduction of VI with sodium borohydride, the maximum at 320 mµ disappeared, and new maxima at 240 mµ and 286 mµ (low intensity) appeared.

In order to investigate possibilities of three partial structures (C, D and E), ultraviolet spectra of the derivatives of I were compared with those of model compounds, p-amino-benzoylelacetone and ethyl p-aminobenzoyleacetate, of which syntheses are to be reported in another paper.

Firstly, the possibility of the β-diketone structure (C) was excluded, because p-amino-benzoylelacetone (H₂SO₄ salt) exhibited absorption maximum at 353 mµ and p-acetylamino-benzoyleacetone at 330 mµ. The second possibility of the β-ketoester structure (D) could not be excluded rigorously by the fact that ethyl p-aminobenzoyleacetate and its acetyl derivative exhibited maxima at 322.5 mµ and 292 mµ respectively. The absence of such ester bonding was confirmed as follows:

Trichomycin A was hydrogenated with Adams’ catalyst in glacial acetic acid containing acetic anhydride, then reduced by sodium borohydride and subjected to alkaline hydrolysis.

The absorption intensity of maximum at 245 mµ of the product was nearly identical with that of starting material of the alkaline treatment, showing no hydrolysis occurred.
as regards the assumed ester bonding.

Thus the existence of the grouping $E$, which gives $p$-aminoacetophenone by retroaldolisation was deduced, and also supported by infrared spectra of $I$ and its derivatives as well as by ultraviolet spectra. Oxidation of $I$ with chromium trioxide gave crystalline compound ($IX$) which was assigned tentative formula $C_{21}H_{27}O_{4}N$. Alkaline degradation of $IX$ gave $p$-N-acetylaminoacetophenone, and ozonolysis of $IX$ gave glyoxal, which was characterized as 2, 4-dinitophenylhydrazone. These results, coupling with data of ultraviolet (223 m$\mu$ and 287 m$\mu$) and infrared absorption, proved the existence of the grouping $E$ (as N-acetyl form) and $-\text{CH} = \text{CH} - \text{CHO}$ in $IX$. The attachment of this moiety to original molecule will be discussed in the Section V.

IV. Other Functional Groups

1. $\beta$-Diketone

Perhydrotrichomycin A, $IV$ which absorbed 11 molar equivalents of hydrogen, had no characteristic absorption in the ultraviolet under neutral and acidic conditions. However the strong absorption occurred at 277 m$\mu$ in alkaline solution (Fig. 6) and this disappeared by acidification. When $IV$ was reduced with sodium borohydride, the reduced product ($X$) had no characteristic absorption even under alkaline conditions. These results indicated the existence of an enolizable group, which was supported by the positive results for bromine decolorization and ferric chloride test. The evidence of its being $\beta$-diketone, not $\beta$-ketoester, was established by the absence of decarboxylation upon acidification after alkaline hydrolysis.

Perhydrotrichomycin A, $IV'$ which absorbed 7 molar equivalents of hydrogen, also had the strong absorption at 272 m$\mu$ in alkaline methanol besides a maximum at 329 m$\mu$. While the former changed as above according to pH of the solution, the latter did not, showing that the $\beta$-diketone system did not conjugate with the $p$-aminobenzoyl group (Fig. 4). The $\beta$-diketone system in $I$ was also indicated by comparing its ultraviolet spectrum in methanol with that in alkaline methanol (Fig. 7).

Fig. 6. Ultraviolet spectrum of perhydrotrichomycin A ($IV$) in (1) water; (2), (1)+1 drop of 2N NaOH; (3), (2)+2 drops of 2N NaOH.

Fig. 7. Comparison of ultraviolet spectrum of trichomycin A in methanol (broken line) with that in methanolic 0.2N NaOH.
Table 1. pKa' values

<table>
<thead>
<tr>
<th></th>
<th>In 66% dimethylformamide</th>
<th>In water</th>
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<tbody>
<tr>
<td></td>
<td>-COOH</td>
<td>-NH(_2)</td>
</tr>
<tr>
<td>Trichomycin A (I)</td>
<td>6.1</td>
<td>9.0</td>
</tr>
<tr>
<td>Trichomycin A treated with NaBH(_4) (II)</td>
<td>6.1</td>
<td>9.1</td>
</tr>
<tr>
<td>Acetylated trichomycin A (III')</td>
<td>6.75</td>
<td></td>
</tr>
<tr>
<td>N-Acetyl-perhydrotrichomycin A (XI)</td>
<td>6.65</td>
<td></td>
</tr>
<tr>
<td>Acetylated perhydrotrichomycin A (VII)</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>Acetylated perhydrotrichomycin A treated with NaBH(_4) and NaOH</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>Perhydrotrichomycin A treated with HIO(_4) and O(_2)</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Perhydrotrichomycin A treated with NaBH(_4) (X)</td>
<td>4.90</td>
<td></td>
</tr>
<tr>
<td>n-Valeric acid</td>
<td>7.3</td>
<td>4.86</td>
</tr>
<tr>
<td>(\gamma)-Amino butylic acid</td>
<td>6.0</td>
<td>10.4</td>
</tr>
<tr>
<td>(d)-Glucosamine</td>
<td>8.1</td>
<td></td>
</tr>
</tbody>
</table>

2. Carboxyl group

Trichomycin A (I) and most of its derivatives were insoluble in water, and therefore the measurements of pKa' values shown in Table 1 were carried out in 66% dimethylformamide.

Trichomycin A had two pKa's, 6.1 and 9.0, and they unchanged upon reduction with sodium borohydride, indicating that they did not originate from \(\beta\)-diketonic group. On the other hand, acetylated trichomycin A (III') and N-acetyl perhydrotrichomycin A (XI) had one pKa, 6.75 and 6.65 respectively, showing that 9.0 of I arised from amino group. Therefore, 6.1 of I and 6.75 of III' were assigned to pKa's of a carboxyl group.

In addition, when perhydrotrichomycin A (IV) was reduced with sodium borohydride, pKa' of the product was 4.9 in water, corresponding to 4.86 of a model. Thus the presence of the carboxyl group is apparent, coupling with the band at 1708 cm\(^{-1}\) in the infrared spectum of I.

3. Lactone

The infrared absorption of I possesses a band at 1734 cm\(^{-1}\) characteristic of an ester or lactone carbonyl grouping. When I was backtitrated with HCl after alkaline hydrolysis, a neutral equivalent was roughly one half that of I, indicating the presence of a potential acidic group. When XII obtained by the NaBH\(_4\) reduction of the acetylated perhydrotrichomycin (VII) was subjected to saponification, as already mentioned in Section III, the product recovered by acidication had approximately the same extinction coefficient value as that of the starting material, showing that there was no cleavage of the molecule upon the saponification. Therefore the hydrolysable group in I was deduced to be a lactone.

4. The grouping F containing \(\alpha\)-glycol

Perhydrotrichomycin A consumed 2 mols of periodic acid within 2 hours. The absence of formic acid in the reaction mixture and the positive iodoform reaction for I suggested that the mycosamine part was in the type of furanos. Therefore, one mol of
periodic acid must be consumed by the mycosamine part. When IV was oxidized with periodic acid, the product exhibited a maximum at 237 mµ in acidic condition. The occurrence of such chromophore suggested that the carbonyl group formed by the oxidation caused β-hydroxyl group to eliminate. Coupling with the evidence of the absence of tert-hydroxyl group based on infrared absorption of the completely acetylated derivatives of I or VII (Figs. 1 and 8), the grouping F was supported by the following result: Ozonolysis of this oxidation product gave methyglyoxal which was characterized as 2,4-dinitrophenylhydrazone.

\[
\begin{align*}
\text{H} & \quad \text{H} & \quad \text{H} & \quad \text{H} \\
\text{OH} & \quad \text{OH} & \quad \text{CH}_3 & \quad \text{OH} \\
\text{F}
\end{align*}
\]

V. Skeletal Structure

There are two possibilities in the ways of the attachment of the polyene: (1) Both sides of the polyene grouping bond with the residual part of the molecule. (2) The polyene grouping divides the molecule into two parts.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Oxygen</th>
<th>Carbon</th>
<th>Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Diketone</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>C19-Moiety</td>
<td>2</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>Lactone</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Carboxyl</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>(A)+mycosamine</td>
<td>6</td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td>(F)</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Epoxide</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncounted above</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>61</td>
<td>2</td>
</tr>
</tbody>
</table>
In the case (2), the cleavage with ozone should divide the molecule into two fragments with the exception of the products from polyene group itself. However any lower carbonyl compounds and acids were not detected and any evidence of such cleavage could not be obtained. Therefore, the cyclic attachment appeared to be more probable.

Upon the treatment of I with acetic acid, furthermore, it was found that the ultraviolet spectrum of I changed to the all-trans type in which the peak at the longest wavelength is the highest (Fig. 9). Since the ring appears to be unable to open in the used condition, the ring must be large enough to keep the heptaene system unhindered in order to make such isomerization possible. The inspection of models shows that the ring must be of at least 31 atoms.

Fig. 10

All the functional groups in the molecule were clarified as shown in Table 2 and Fig. 10 (Three atoms of the C\textsubscript{19} moiety may overlap on the grouping A, and one or two hydroxyl in the last may attach to C\textsubscript{19}-moiety. The existence of an epoxide group was suggested only by the test with sodium thiosulfate.)

Experimental

Melting points were uncorrected. Unless otherwise stated, the ultraviolet spectra were measured on Beckman DK2 in methanol and infrared spectra were measured in KBr on Hilgar H800 and Kohken IR S instrument, equipped with rosksalt prisms. Crystalline trichomycin A was used as starting materials to prove that the products obtained did not originate in impurities. The mixture purified as described in the previous paper was termed as 'purified trichomycin' and used in some cases because same conjugate systems and aminosugar exist in both trichomycin A and trichomycin B. Amorphous trichomycin A isolated by counter-current distribution was termed as 'trichomycin A'.

Reduction of trichomycin A (I) with sodium borohydride (II)

Trichomycin A (0.12 g) was suspended in 5 ml of water, dissolved by adding 0.02 g of sodium borohydride and allowed to stand overnight at room temperature. Precipitate formed by neutralization at pH 6.8 with 1 N HCl was collected and washed with water and acetone by centrifugation, and again washed with acetone and ether by filtration. A pale yellow substance (II) showing amphoteric property in water was obtained. Yield 0.9 g. Two mg of II was placed in Ckaio machine using the solvent system, chloroform-methanol-pH 8.5 borate buffer (2 : 2 : 1). Distribution curves were traced by the extinction at 385 m\mu and the absorption curve of the peak No. 54 in 61 transfers was measured.
Ultraviolet maxima: 345, 364.5, 384.5 and 409 m\(\mu\) in the upper phase of chloroform-methanol-8.5 borate buffer (2:2:1).

Acetylation of I (III)
Trichomycin A (1.25 g) was suspended in 10 ml of acetic anhydride and dissolved by adding 10 ml of pyridine. The mixture was heated at 60°C for 2 hours and then concentrated to 1/4 of its volume under reduced pressure. The concentrate was triturated with 200 ml of ether, and the precipitate was collected and washed with ether to yield 1.32 g of the completely acetylated trichomycin A (III).

Infrared and ultraviolet spectra are shown in Fig. 1 and Fig. 2 respectively.

Acetylation of I (III')
Trichomycin A (1.23 g) was dissolved in a mixture of 10 ml of glacial acetic acid and 10 ml of acetic anhydride, and allowed to stand for 1 hour at room temperature. The mixture was poured into 200 ml of ether, and the material precipitated was collected by filtration and washed with ether to yield 1.41 g of the acetylated trichomycin A (III'). The material thus obtained was not homogeneous, but useful for the purpose of the alkaline degradation, pKa measurements and so on. The average numbers of acetylated groups were 5.1 in two samples.

Oxidation of I with chromium trioxide (2, 4, 6, 8-decatetraene 1, 10 dial)
Purified trichomycin (4.3 g) was dissolved in 120 ml of glacial acetic acid containing 20 ml of acetic anhydride, and the solution was filtered after addition of 30 ml of benzene. Each 0.7~1.2 ml of 40 ml aqueous solution containing 2 g of chromium trioxide and 3 g of potassium bichromate, was added into each 10~15 ml of the trichomycin solution at room temperature, stirred for 40~60 seconds, and then each of the reaction mixture was diluted with 30~40 ml of water. The combined solution was extracted with each 100 ml of benzene three times. The benzene extract was dried with anhydrous sodium sulfate and concentrated under reduced pressure. Ten ml of petroleum-ether was added to about 3 ml of the concentrate and the solution chromatographed on alumina. The eluate of petroleum ether-ether (4:1) was re-chromatographed on alumina. One re-crystallization gave 15 mg of crystal, m.p. 149~152°C.

Infrared bands: 1675, 1629, 1400, 1187, 1146, 1121, 1025 and 999 cm\(^{-1}\).

Ultraviolet maxima: 319, 337 and 354 m\(\mu\) in ether. After shaking with a small amount of sodium borohydride, the solution exhibited maxima at 273, 296 and 310 m\(\mu\).

Oxidation of I with chromium trioxide (C\(_{19}\)-moiety, IX)
Purified trichomycin (2 g) was dissolved in 120 ml of glacial acetic acid containing 40 ml of acetic anhydride, and then 25 ml of the aqueous solution containing chromium trioxide (1 g) and potassium bichromate (1 g) was added at room temperature, and 5 minutes later 200 ml of water was added. The mixture was extracted with 100 ml of benzene thrice, and the aqueous layer was extracted successively with ether for 70 hours. The ether layer was neutralized with sodium bicarbonate solution to pH 6.5, and evaporated under reduced pressure. After washing with 40 ml of petroleum-ether, the residue was extracted with a mixture of ethyl acetate-benzene (2:1) and the extract was evaporated under reduced pressure. Two recrystallizations of the residue from a mixture of chloroform-benzene (5:95) yielded 45 mg of fine crystal, m.p. 136~137°C.

Ultraviolet maxima: 223 (E 825) and 286 m\(\mu\) (E 707).

Infrared bands: 3313, 2900, 2860, 1665, 1595, 1523, 1405, 1372, 1270, 1218, 1181, 1120, 1085, 1035, 995, 965, 880 and 830 cm\(^{-1}\).

Anal.: obsd. C 68.92, H 7.53, N 3.82 calcd. for CH\(_3\)CO-C\(_{19}\)H\(_{2}\)O\(_{N}\)•½H\(_{2}\)O C 69.32, H 7.65, N 3.82

Alkaline degradation of I (The detection of octaenal-chromophore)
Trichomycin A (1.0 g) was dissolved in 20 ml of 2 n H\(_2\)OH and allowed to stand overnight at room temperature. The solution was extracted with each 20 ml of ether (200 ml), the combined ether extract was washed with 50 ml of 0.33 n HCl and with 30 ml of water, dried with anhydrous sodium sulfate, concentrated to about 1/5 of its volume, and chromatographed on alumina. The first yellow band of the chromatogram eluted with a mixture of ether and acetone (2:1) was evaporated to leave the orange-yellow residue, which was dissolved in ether and subjected to ultraviolet measurement. The spectrum of the ether solution was shown in Fig. 3a.

The ether solution was evaporated under reduced pressure and the spectrum of the residue was measured in the methanol solution containing a small amount of sodium borohydride using the same solution as reference (Fig. 3b).
Alkaline degradation of I (p-Aminoacetophenone)

Purified trichomycin (500 mg) was dissolved in 50 ml of 1 N NaOH, allowed to stand for 3 days under nitrogen atmosphere and extracted with ether. When the extract was evaporated under reduced pressure, 38 mg of crude crystal was formed. After decolorization with charcoal, one recrystallization from ether gave pale yellow needles, m.p. 98~102°C. Identity with authentic material was confirmed by infrared and mixed melting point.

Evidence of the existence of p-aminophenyl group in crystalline trichomycin A was established as follows: The crystal (1.66 mg) was dissolved in 1 ml of 1 N NaOH, allowed to stand at room temperature for 2 days and extracted with 9 ml of ether. The ether extract contained 63 mcg (34% of 1 molar equivalent) of p-aminoacetophenone on the basis of ultraviolet absorption.

Acetylated degradation of acylated trichomycin A, III' (p-acetylaminoacetophenone)

Acetylated trichomycin A (III', 500 mg) was dissolved in 1 N NaOH, allowed to stand overnight, and extracted with ether. Crude crystal (42 mg) was obtained. One recrystallization from ethanol gave colorless needles, m.p. 158~161°C. Identity with authentic material was confirmed by infrared absorption and mixed melting point.

Hydrogenation of I (Perhydrotrichomycin A, IV which absorbed about 11 molar equivalent of hydrogen)

Trichomycin A (2.0 g) was dissolved in glacial acetic acid (50 ml) and 500 mg of Adams' catalyst was added. The mixture was stirred at 15°C under 20 mmHg pressure of hydrogen for 4 hours at which time the hydrogenation stopped and 385 ml of hydrogen (94% of 11 molar equivalents) had been absorbed. After removal of catalyst by filtration and concentration of acetic acid, an appropriate amount of decolorizing charcoal and ether were added to precipitate a small amount of colored material. Then the solution was filtered and 200 ml of ether was added. The precipitate formed was collected, washed with ether and dried in vacuo. White amorphous powder (1.72 g) was obtained.

Ultraviolet spectrum in Fig. 6.

Hydrogenation of I (Perhydrotrichomycin A, IV' which absorbed about 7 molar equivalents of hydrogen)

Trichomycin A (1.2 g) was dissolved in glacial acetic acid (50 ml) and 120 mg of platinum oxide was added. The mixture was stirred at 15°C under hydrogen atmosphere. One hundred and sixty ml hydrogen were absorbed for 10 hours. The subsequent procedure was carried out in the same way as above to yield 1.8 g of white amorphous powder.

Ultraviolet spectrum in Fig. 4.

Hydrogenation of I in acetic acid containing acetic anhydride (VII)

Purified trichomycin (2 g) was dissolved in 50 ml of acetic acid-acetic anhydride (9 : 1) and hydrogenated as above absorbing 257 ml of hydrogen.

Ultraviolet maximum : 286 mµ (E 140)

Acetylation of VII (Completely acetylated perhydrotrichomycin)

In 20 ml of acetic anhydride-pyridine (1 : 1), 1.9 g of VII was dissolved, allowed to stand overnight and heated on a water-bath (80°C) for 8 hours. The mixture was evaporated under reduced pressure. The residue was dissolved in 10 ml of chloroform, and diluted with 10 ml of benzene. After removal of precipitate, the solution was chromatographed on alumina. The chloroform eluate was evaporated under reduced pressure to yield 1.0 g of colorless glass.

Ultraviolet maximum : 299 mµ (It may be due to the enolization of β-diketonic group or the carbonyl group of p-aminobenzoyl structure that the maximum was shifted to longer wavelength compared with that of p-acetylaminoacetophenone.)

Infrared spectrum in Fig. 9.

Reduction of IV with sodium borohydride (X)

One g of IV was dissolved in 5 ml of water and added with 0.5 g of sodium borohydride. The solution was allowed to stand overnight at room temperature, acidified with acetic acid and passed through IRC 50 column (200 mesh). The column was washed with 400 ml of 0.5 N HCl and 200 ml of 1 N HCl to remove boric acid and NaCl. The reduced product was eluted with 50% acetic acid and the eluate was evaporated under reduced pressure to leave 0.53 g of glassy residue. No characteristic absorption was observed in the concentration of 0.5 mg/ml of water. The value pKa' is shown in Table 1.

Hydrogenation of I in methanol containing acetic anhydride (N-acetyl perhydrotrichomycin A, XI)

Trichomycin A (234 mg) was dissolved in 20 ml of methanol containing 0.2 ml of acetic anhydride and hydrogenated with platinum oxide as above. The resulted solution was added with 10 ml of
water, allowed to stand overnight, evaporated to dryness and washed with acetone and ether to yield 214 mg of white amorphous substance.

Ultraviolet maximum: 246 mμ (E 29) (In this case, the hydrogenation of the carbonyl group of aminobenzyl structure occurred.)

Infrared spectrum in Fig. 11.

**Fig. 11**

Reduction of VII with sodium borohydride (XII)

Acetylated perhydrotrichomycin A (1.8 g) was suspended in 30 ml of water, and 0.8 g of sodium borohydride was added. The solution was allowed to stand overnight, neutralized to pH 6.8 with 1 N HCl and the material precipitated was centrifuged. The precipitate was then collected by filtration, washed with water and acetone, and dried in vacuo to yield 1.2 g of white solid.

Ultraviolet maximum: 245 mμ (E 129)

Alkaline treatment of XII (XIII)

In 5 ml of 1 N NaOH, 0.4 g of XII was dissolved and warmed at 80°C on a water-bath for 1 hour. After cooling the solution was neutralized with 1 N HCl at pH 6.8 to form the precipitate, which was collected, washed with water and acetone by centrifugation, and dried in vacuo to yield 0.28 g of white solid.

Ultraviolet maximum: 245 mμ (E 120)

Dinitrophenylation of IV (N-DNP-perhydrotrichomycin A)

Perhydrotrichomycin (0.80 g) was dissolved in 100 ml of 50% ethanol and 1.0 g of sodium bicarbonate was suspended. The solution was treated with 50 ml of ethanol containing 2,4-dinitrofluorobenzene (0.30 g), and stirred for 10 hours at room temperature. The solution was acidified with acetic acid, and concentrated under reduced pressure. The concentrate was extracted with 50 ml of ethyl acetate twice and the combined extract was washed with 25 ml of water twice, and the ethyl acetate layer was concentrated to yield syrupy residue. After triturating with ether and drying in vacuo, 0.78 g of yellow powder was obtained.

Ethanolysis of N-DNP-perhydrotrichomycin A (Ethyl 1 N-DNP-mycosaminide, V)

N-DNP-perhydrotrichomycin A (0.78 g) was dissolved in 35 ml of ethanol and 3.3 ml of concentrated HCl was added. The solution was boiled for 2 hours. After cooling, the solution was diluted with 30 ml of water, extracted with 60 ml of ether thrice, and then ether extract was washed with dilute sodium bicarbonate solution and water, dried with anhydrous sodium sulfate and then evaporated to dryness in vacuo. Ether extract of the residue was chromatographed on alumina, and eluate of ether containing 2.5% of methanol was evaporated to give 38 mg of yellow crystal. Three recrystallizations from benzene gave prisms, m.p. 80~82°C. Identity with ethyl N-DNP-mycosaminide obtained from pimaricin in the same procedure as above, was confirmed by infrared and mixed melting point.

Ultraviolet maximum: 351.5 mμ (E 24500)

Infrared bands: 3462, 3330, 2977, 2875, 1614, 1518, 1476, 1420, 1333, 1227, 1125, 1103, 1074, 1052, 978, 921, 843, 835, 823, 780, 760 and 738 cm⁻¹

Anal. obsd.: C 55.28, H 5.87, N 9.66
calcd. for C₁₄H₁₉O₆N₂·C₄H₄: C 55.17, H 5.79, N 9.65
Hydrolysis of IV (Mycosamine HCl)
Perhydrotrichomycin (4.3 g) was dissolved in 90 ml of 6 N HCl, and allowed to stand for 1 month at room temperature under nitrogen atmosphere. Dark brown precipitate formed was removed by decantation and the hydrolysate was evaporated in vacuo, and the syrupy residue was redissolved in 10 ml of water and evaporated. This procedure was repeated twice more. The residue was dissolved in 15 ml of water and passed through Amberlite IR 120-column, and eluted with 1 N HCl after washing with water. The fraction positive for triphenyltetrazolium chloride was gathered and concentrated. The concentrate was chromatographed on paper powder using the upper phase of solvent system, acetic acid - n-butanol-water (4: 1: 5), and the fractions positive for both triphenyltetrazolium chloride and ninhydrin, were extracted with methanol, evaporated, redissolved in methanol and precipitated by addition of acetone. The white hygroscopic powder (0.02 g) was obtained, and showed the same Rf-values as below.

Paper partition chromatography of the hydrogenolysis product of I (Mycosamine HCl)
The etheral wash solution in the preparation of perhydrotrichomycin A, IV (see above) was concentrated to leave syrupy residue and 30 ml of ether was added. After removal of precipitate by filtration, the filtrate was concentrated again to yield syrupy residue. This was spotted on paper and subjected to paper partition chromatography using the following solvent systems.

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>Syrupy residue</th>
<th>Mycosamine obtained from pimaricin</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Butanol-acetic acid-water (4: 1: 5)</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td>n-Butanol-diosane-2 N aqueous ammonia (4: 1: 5)</td>
<td>0.42</td>
<td>0.42</td>
</tr>
<tr>
<td>Pyridine-ethyl acetate-acetic acid-water (5: 5: 1: 3)</td>
<td>0.48</td>
<td>0.48</td>
</tr>
<tr>
<td>Sec.-Butanol (saturated with water)</td>
<td>0.47</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Acetylation of acetylated perhydrotrichomycin (Mycosamine acetate)
Acetylated perhydrotrichomycin (490 mg) was dissolved in 30 ml of a mixture of glacial acetic acid-acetic anhydride (7: 3) containing 2% sulfuric acid, and allowed to stand for 6 days. After removal of brown precipitate, the solution was diluted with water, and then extracted with chloroform. The chloroform layer was washed with water and evaporated to dryness. The residue was dissolved in benzene and chromatographed on alumina. Chloroform eluate was evaporated to yield 15 mg of colorless glassy substance, which was positive for triphenyltetrazolium chloride reactions. The characteristic absorption was not observed in the ultraviolet.

Infrared bands: 3468, 2988, 1753, 1683, 1513, 1425, 1371, 1216, 1145, 1049, 1020, 967, 933, 911, 893, and 836 cm⁻¹ in chloroform.

Ozonolysis of III'
Three to five % ozone was passed through the solution of III' (2 g) in 50 ml of acetic acid-acetic anhydride (9: 1), the decolorized solution was added with 10 ml of water and concentrated to leave syrupy residue, which was triturated with ether, ethyl acetate and again ether to yield 1.3 g of white powder.

Infrared spectrum: 1674 (broad), 1597, 1515, 1408, 1373, 1316, 1290, 1230 (broad), 1180, 1039, and 998 cm⁻¹.

Ultraviolet spectrum: Fig. 5.

Oxidation of IV with sodium periodate and ozonolysis of the product
Perhydrotrichomycin A (IV, 2 g) was dissolved in 40 ml of pH 4.5 acetic acid buffer and added with 20 ml of the same buffer solution containing 0.7 g of sodium periodate. The precipitated solid was collected by filtration and washed with water, and dissolved in a small amount of dilute sodium bicarbonate solution. Reprecipitation by adding an appropriate amount of acetic acid was followed by washing with water and drying in vacuo to yield 1.4 g of colorless solid. The alkaline solution of this solid exhibited maximum at 270 μ and besides maximum at 329 μ (low) and by acidification the former disappeared and the maximum at 237 μ appeared. The oxidation product obtained as above (1.35 g) was dissolved in 40 ml of acetic acid-acetic anhydride (9: 1) and ozonized. The solution was diluted with 40 ml of water and centrifuged after being kept for 12 hours. The aqueous solution was distilled and the distillate was collected in the 2,4-dinitrophenylhydrazine solution. The precipitate was collected by filtration and recrystallized from nitrobenzene to yield 40 mg of crystal, which was identified with authentic methylglyoxal 2,4-dinitrophenylhydrazone by comparing the infrared spectrum and mixed melting point. The precipitate obtained by the centrifugation was dissolved in 15 ml of methanol and evaporated to dryness and redissolved in methanol and concentrated to leave about 2 ml of syrup. The residue was triturated with 30 ml of ether, recovered by filtration.
and dried to give 1.02 g of solid. The solution of this material (6.26 mg) in 10 ml of water exhibits no characteristic absorption in the ultraviolet and a maximum at 273 m\(\mu\) appeared by adding alkali. The \(pK_a'\) is shown in Table 1.

The \(pK_a'\) measurements

The \(pK_a'\)s were measured by potentiometric titration using pH meter Beckman model G and ultramicroburette. For example, 15.80 mg of crystalline trichomycin A was dissolved in a mixture of 0.0469 \(n\) HCl (1 cc) and 80% dimethyl formamide (4 ml), and titrated with 0.912 \(n\) NaOH. A neutral equivalent of 1230 was obtained. The \(pK_a'\) values are shown in Table 1.

Crystalline trichomycin A (15.69 mg) was measured in a micro-glass tube and 0.113 ml of 0.932 \(n\) NaOH was added using an ultramicro-burette. Then 0.5 ml of water was added to wash off the point of burette, and the solution was warmed on a water-bath (60°C) for 1 hour after sealing the tube. Then the tube was carefully crushed in a small beaker, added with 4 ml of 80% dimethylformamide and titrated with 1.751 \(n\) HCl. A neutral equivalent of 470 was obtained by comparing with the blank procedure.

Oxidation with periodical acid

Methyl N-DNP-mycosaminide (6.43 mg) was dissolved in 1 ml of methanol, added with 3 ml of pH 4.5 acetate buffer and 4 ml of 0.02 \(n\) periodic acid, and finally diluted with the acetate buffer to exactly 10 ml. Each 1 ml of the mixed solution was pipetted out after 2 hours, 5 hours and so on, to be titrated with 0.005 \(n\) iodine solution. Upon oxidation of perhydrotrichomycin A, methanol was not added. The oxidation of I was carried out in alkaline media because of insolubility of I, and 2 mols were consumed within 50 hours.

<table>
<thead>
<tr>
<th></th>
<th>2</th>
<th>5</th>
<th>8</th>
<th>22</th>
<th>46</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl N-DNP-glucosaminide</td>
<td>0.84</td>
<td>1.10</td>
<td>1.53</td>
<td>1.96</td>
<td>2.53</td>
<td></td>
</tr>
<tr>
<td>N-DNP-glucosamine</td>
<td>2.63</td>
<td>3.41</td>
<td>3.67</td>
<td>4.50</td>
<td>5.13</td>
<td>5.43</td>
</tr>
<tr>
<td>Methyl N-DNP-mycosaminide</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Perhydrotrichomycin A</td>
<td>1.91</td>
<td>2.24</td>
<td>2.36</td>
<td>2.68</td>
<td>3.15</td>
<td>3.70</td>
</tr>
</tbody>
</table>

Treatment of I with active manganese dioxide

Ten mg of I was dissolved in 5 ml of 70% aqueous acetone and filtered. The filtrate was stirred with 50 mg of active manganese dioxide for 1 hour, filtered and subjected to the measurement of the ultraviolet spectrum, which was identical with that of starting material.

Treatment of I with acetic acid

Thirty mg of I was dissolved in 10 ml of 50% acetone at 60°C and allowed to stand for 30 minutes. After cooling, the precipitate formed was collected by filtration, washed with acetone and ether and dried to yield 22 mg of yellow powder.

Ultraviolet spectrum in Fig. 9.

Infrared bands: 3490, 2980, 1734, 1708, 1634, 1597, 1568, 1444, 1403, 1383, 1321, 1251, 1223, 1176, 1130, 1100, 1068, 1038, 886, 848 and 769 cm\(^{-1}\).

Summary

As shown in Fig. 10, heptaenic part, mycosamine, \(p\)-aminobenzoyl, \(\beta\)-diketonic, carboxyl, lactonic and \(\alpha\)-glycol group in trichomycin A were elucidated and its macrocyclic structure was suggested.

Acknowledgement

The author wish to express his sincere thanks to Prof. YOSHIKASA HIRATA for his kind guidance and encouragement, to Dr. MASAO OHARA for his encouragement and kindness, and to Mr. KIYOSHI HATTORI for his assistance. The author is also grateful to Dr. TADASHI KARASAKI for measurement of potency and to Mr. SHIGETERU OHTA for microanalyses.

Reference