PHYSICAL AND CHEMICAL CHARACTERISTICS AND STRUCTURE OF CARMINOMYCIN, A NEW ANTITUMOR ANTIBIOTIC

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In the course of our screening program we have isolated from the culture of *Actinomadura carminata* carminomycin, a new anthracycline antibiotic which possessed strong antitumor activity in animal experiments. It is produced as a mixture of several active components: the more interesting ones are components 1, 2 and 3. Components 2 and 3 can be transformed by means of mild hydrolysis into carminomycin 1, which is obtained as a red crystalline hydrochloride C_{60}H_{12}O_{36}N.HCl. The ultraviolet and visible spectra resemble that of daunomycin but are different.

Carminomycin 1 on hydrolysis with 0.1N hydrochloric acid, yields daunosamine and an aglycone, carminomycinone. The structure of aglycone and carminomycin 1 was determined by physical-chemical methods.

Carminomycin was isolated from the mycelium of *Actinomadura carminata* in the form of a complex preparation, which contains seven colored components, five of which are biologically active. Carminomycins 2 and 3 are produced preferentially. The other components of the carminomycin complex are present in negligible amounts. By extraction and chromatography on silicic acid from chloroform-benzene-methanol (7 : 3 : 3), three of the more active components were separated from the less active ones. Fig. 1 shows the separation of the complex preparation by means of thin-layer chromatography on silica gel G. The complex preparations contain 50% of carminomycin 2 and 30% of carminomycin 3. All three active components were obtained in the form of free bases. Carminomycin 1, which presents the greatest interest from the chemotherapeutic point of view, was also obtained in the form of a hydrochloride crystallized from a mixture of ethanol and benzene. The crystalline salt is soluble in water and methanol, and insoluble in other organic solvents. The biological assays determined by the agar-diffusion method with *Bacillus mycoides* as test organism, give for carminomycins 1, 2 and 3, respectively, 1,000 u/mg, 580 u/mg and 730 u/mg. In Table 1 are given certain physico-chemical properties of the carminomycins. As can be seen, carminomycin 3 is more lipophylic than carminomycins 1 and 2. This follows from comparison of the coefficients of distribution in the system chlo-
Table 1. Comparison of physico-chemical properties of carminomycins 1, 2 and 3.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Elementary analysis (%)</th>
<th>Formula</th>
<th>Distribution factor</th>
<th>UV absorption maxima (nm)</th>
<th>Specific rotation [a]_D^0</th>
<th>Equivalent weight</th>
<th>pK_a</th>
<th>pK_a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carminomycin 1</td>
<td>C 56.69, H 5.80, N 2.75, Cl 5.36</td>
<td>C_{26}H_{17}O_{10}N·HCl</td>
<td>0.06</td>
<td>236, 255, 462, 478, 492, 510, 525</td>
<td>300 +289°</td>
<td>286</td>
<td>8.00</td>
<td>10.16</td>
</tr>
<tr>
<td>Carminomycin 2</td>
<td>C 60.30, H 6.71, N 2.56</td>
<td></td>
<td>1.5</td>
<td>the same</td>
<td>227 +565°</td>
<td>351</td>
<td>8.00</td>
<td>10.10</td>
</tr>
<tr>
<td>Carminomycin 3</td>
<td>C 57.30, H 6.21, N 2.56</td>
<td></td>
<td>14.0</td>
<td>the same</td>
<td>220 +133°</td>
<td>371</td>
<td>8.00</td>
<td>10.10</td>
</tr>
</tbody>
</table>

Carminomycins possess the ultraviolet and visible spectra, typical of anthracyclines. These spectra are closest to the spectra of daunomycin, though different (Fig. 2). Specific absorption at 492 nm is practically the same for carminomycins 2 and 3, but is 80 units less than the specific absorption of carminomycin 1.

IR spectra of carminomycin 1 and daunomycin, shown in Fig. 3 also demonstrate the differences of these two antibiotics. Carminomycins 1, 2 and 3 have different specific optical rotation. The equivalent weight by potentiometric titration is less for carminomycin 1 than for carminomycins 2 and 3. The same conclusion can be made from differences in specific absorption of carminomycins. The pK_a figures show that amino and phenolic groups are present in carminomycins.

Carminomycins 2 and 3 can be transformed into carminomycin 1 by means of mild hydrolysis with the liberation of carbohydrate fragments, similar to the conversion of rubomycin B into rubomycin C. Carminomycins 4, 5 and 6 in the complex preparation in small quantities have been isolated by chromatography on silicic acid. Carminomycin 4 is a brick-red amorphous substance, easily soluble in most of organic solvents. It is biologically active (450 u/mg). It contains 2.4% nitrogen and is not changed under mild hydrolysis.

Carminomycin 5 is a dark-red amorphous substance, easily soluble in organic solvent assays 350 u/mg, contains 2.17% nitrogen and does not change under mild hydrolysis.

Carminomycin 6 is a crystalline substance without nitrogen. It is also formed during...
Table 2. Comparison of physico-chemical properties of carminomycinone, daunomycinone and their derivatives.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>CH₃O- (%)</th>
<th>Melting point (°C)</th>
<th>Specific rotation [α]D²⁰</th>
<th>UV absorption (nm) (EtOH)</th>
<th>E₁% cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carminomycinone</td>
<td>C₂₀H₁₀O₈</td>
<td>none</td>
<td>274</td>
<td>+272° (c 0.1, dioxane)</td>
<td>492</td>
<td>400</td>
</tr>
<tr>
<td>Daunomycinone</td>
<td>C₂₁H₁₈O₈</td>
<td>7.53</td>
<td>214</td>
<td>+193° (c 0.1, dioxane)</td>
<td>495</td>
<td>310</td>
</tr>
<tr>
<td>Carminomycinone acetate</td>
<td>C₂₀H₁₀O₁₀</td>
<td>none</td>
<td>190</td>
<td>-40° (c 0.11, chloroform)</td>
<td>346</td>
<td>244</td>
</tr>
<tr>
<td>Daunomycinone acetate</td>
<td>C₂₃H₂₀O₁₂</td>
<td>5.48</td>
<td>225</td>
<td>-95.5° (c 0.11, chloroform)</td>
<td>379</td>
<td>116</td>
</tr>
<tr>
<td>Carminomycinone tetramethyl</td>
<td>C₂₄H₂₄O₈</td>
<td>28.17</td>
<td>188</td>
<td>+266° (c 0.1, dioxane)</td>
<td>376</td>
<td>197</td>
</tr>
<tr>
<td>Daunomycinone trimethyl</td>
<td>C₂₄H₂₄O₈</td>
<td>28.17</td>
<td>193</td>
<td>+181° (c 0.1, dioxane)</td>
<td>376</td>
<td>197</td>
</tr>
</tbody>
</table>

Hydrolysis of carminomycins 1, 2, 3, 4 and 5 and therefore represents the aglycone of these antibiotics.

When carminomycin 1 is hydrolyzed (0.1 N hydrochloric acid, 100°C, 30 minutes), it yields a red aglycone and aminosugar (carminosamine) which has positive reaction with ninhydrin. The aminosugar has been isolated from hydrolyzates of carminomycins 1, 2 and 3 by chromatography on Dowex 50×12 (H⁺) with 0.5 N ammonia. The specific rotation of the basic aminosugar [α]D²⁰ 54.6° (c 0.1, water) and chromatography on paper and Silufol plate revealed that carminosamine is identical with daunosamine. The N, O-acetates of the methylglycosides of carminosamine and daunosamine gave identical n.m.r. spectra.

These data show that carminomycin 1 differs from other anthracycline antibiotics in the aglycone.

We prepared crystalline carminomycinone, its acetate and methyl ether and similar derivatives of daunomycinone for comparison. Table 2 shows the physico-chemical properties of carminomycinone and its derivatives. Carminomycinone has no methoxyl groups whereas daunomycinone contains 7.53 % methoxyl, which represents one group per mole of aglycone. The elementary formula of carminomycinone differs from that of daunomycinone by CH₂ and they have the same oxygen content. This indicates that carminomycinone contains a hydroxyl instead of methoxyl.

Fig. 4 shows the IR-spectra of the aglycones. In both compounds the band at 1715 cm⁻¹ (υC=O) is assigned to a COCH₃ group in the hydroaromatic ring. The band at 1625 cm⁻¹ in daunomycinone can be assigned to the carbonyl groups of the anthraquinone, the band at 1580 cm⁻¹ to the aromatic double bond system. The substitution of -OCH₃ group by hydroxyl is accompanied by a shift of 1625 cm⁻¹ band towards lower frequencies (1610 cm⁻¹) hydrogen bonding. This gives superimposition of υC=O and υC-O vibrations in one band at 1610 cm⁻¹.

The difference between carminomycinone and daunomycinone is clear from the NMR-spectra.
of their acetates. The spectrum of carminomycinone acetate (Fig. 5) has three three-proton singlets (δ 2.33, 2.38, 2.45 ppm) which belong to acetoxyls attached to C₄, C₁₁, and C₆ respectively and one six-proton singlet (δ 1.85 ppm) from the acetoxyls attached to C₇ and C₈. The spectrum of daunomycinone acetate lacks the singlet at δ 2.33 ppm and has a three proton singlet at δ 3.92 ppm, corresponding to methoxyl at C₄. Thus carminomycinone differs from daunomycinone by substitution at C₄ with hydroxyl rather than methoxyl.

The methyl ethers of both aglycones contain the same percentage of methoxyl groups and have the same molecular formulas. The NMR-spectra of ethers (Fig. 6) have two three-proton singlets (δ 3.89 and 3.56 ppm), assigned to the methoxyls attached to C₄ and C₇, and one six-proton singlet (δ 4.00 ppm), from the methoxyls at C₆ and C₁₁, confirming the above structural assignment.

The attachment of the sugar in the antibiotic was proven by hydrogenolysis of carminomycin (5% Pd/BaSO₄) with elimination of the hydroxyl and the attached sugar⁶. Hydro-
genation of the aglycone under the same conditions gave a product identical with the product from hydrogenolysis of the antibiotic. The NMR-spectrum of the acetate of the reduced aglycone showed the absence of acetyl at C7 that identifying the hydroxyl involved in the glycosidic bond.

The difference in the melting points of the methyl ethers of carminomycinone and daunomycinone and in the specific rotation of the aglycones and their derivatives implies that the stereochemical properties of aglycones may be different. The stereochemistry of carminomycinone is being investigated at present and will be reported in a separate paper.
Fig. 7 shows a possible scheme for mass-spectral fragmentation of carminomycinone. The molecular ion $m/e$ 384$^+$ (a) in consecutive order loses two molecules of water from elimination of hydroxyls at C7 and C9 with formation of the fragments $m/e$ 366$^+$ (b) and 348$^+$ (c)$^9$. Other assignments can be made for ions at $m/e$ 323$^+$ (b-COCH$_3$ (d)), 333$^+$ (c-CH$_3$ (e)) and 305 (d-H$_2$O (f)), and are confirmed by the corresponding metastable peaks. Therefore, the mass spectrum supports the structure of carminomycinone (Fig. 7a), as a 1, 4, 5-trioxanthraquinone.

Since glycosidic bond is at C7 and carbohydrate fragment is represented by daunosamine, the structural formula of carminomycin 1 may be proposed as shown in Fig. 8.

The study of crystalline carminomycin 1 in animal experiments showed that cancer of praestomach in mice, strain OG-5, is suppressed by 80% with three intravenous injections of half the LD$_{50}$. The bronchogenous lung cancer, strain RL, in experiments on mice, with similar dosages is 95% suppressed by carminomycin. Especially interesting were the results obtained in treating the ascitic form of lymphatic leukemia in mice, strain 1210. In this case the effectiveness of carminomycin 1 is twice as effective as the synthetic antitumor preparation methotrexate. Some of the mice treated with carminomycin 1 survived whereas all mice treated with methotrexate or daunomycin died. In contrast to other antibiotics of this group, carminomycin 1 is very well absorbed from gastroentestinal tract when given orally$^9$.

References
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