Structural studies on a oxohexaene polyene antibiotic (HA-1-92) produced by
Streptomyces CDRIL-312

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Received 9 October 2000; accepted (revised) 12 June 2001

HA-1-92 has been isolated from the culture broth of Streptomyces CDRIL-312 and purified by silica gel column chromatography, followed by preparative TLC. Based on physicochemical properties, spectral studies (UV, IR, FTIR, HPLC, NMR and MS), elemental analysis and comparative data of other oxohexaenes viz. mycoticin A, roflomycin and dermostatin, HA-1-92 is found to be a new oxohexaene polyene macrolide. Its chemical structure has been assigned based on afore mentioned data.

Results and Discussion

During soil screening programme in search of new antifungal antibiotics, a streptomyces culture obtained from CDRI, Lucknow, India named CDRIL-312, was found to produce a polyene macrolide of oxohexaene type and designated as HA-1-92. We have already reported its fermentation, isolation, purification and antimicrobial activity. The liposomal incorporated preparation of HA-1-92 and its effect on aspergillosis has also been reported. This communication reports the chemistry and likely chemical structure of oxohexaene polyene macrolide.

HA-1-92 was obtained as a yellow amorphous powder, m.p. 215-17°C. It was soluble in MeOH, EtOH, BuOH, (CH₃)₂CO, C₂H₅N, DMF and DMSO and insoluble in n-C₆H₁₄, CHCl₃, CCl₄, (C₂H₅)₂O, pH 4.4, [α]D₂⁰ = 89.29. In elemental analysis, it was found that HA-1-92 does not contain nitrogen, as also found by its negative response to ninhydrin colour reaction (Found : C, 67.67; H, 8.29; O, 24.26. C₃₁H₄⁹O₉ requires C, 66.43, H, 7.86; O, 25.71%).
The molecular weight of HA-1-92 was found to be 560 from CI-mass spectra, which showed a molecular ion peak at 561 (M + H). The mass spectral data of HA-1-92 showed the presence of fragment ions at M/z 560(M+), 532, 350, 301, 283, 269, 255, 241, 227, 214, 199, 189, 171, 152, 135, 129, 112, 97, 83, 71, 57 and 50. The mass spectral data can be interpreted as follows. A peak at 350 mass could be due to C_{16}H_{30}O_8. The above mass spectral data of HA-1-92 were similar to the fragmentation pattern observed in other oxohexaene polyene antibiotics, e.g. mycoticin A^8. Roflamycin^9,10 dermostatin^10,11 which also supported the position of lactone group in the molecule. The location of carbonyl functional group and methyl group was assigned after studying the aforementioned fragmentation pattern of the mass spectrum and ^1H NMR spectrum. The molecule thus contain, a large macrolide ring of 29 carbon atoms without any amino sugar or p-aminoacetophenone moiety as supported by the findings of acid and alkaline hydrolysis tests. From the ^1H NMR spectrum, it is observed that the double bonds are endocyclic in nature and are on one side while hydroxyl groups and other functional groups are on the opposite side. In HA-1-92, 6 double bonds and the lactone bond is on one side and 4 hydroxyl groups and a carbonyl group are on the other side. A functional ketone and a methyl group is attached to the main skeleton with no other side chain. Since HA-1-92 does not contain nitrogen as evidenced from its elemental analysis, it is clear that it is an oxohexaene like mycoticin A^8, roflamycoin^9,5 or dermostatin^10,13. In order to substantiate further HA-1-92 as a new oxohexaene it was imperative to compare its physicochemical properties and acute toxicity data with the other reported oxohexaenes mentioned earlier.

Careful evaluation of the present analytical data generated in our laboratory as well as data reported earlier^2 and the data available from the literature^5 (Table I) and also from spectral data of micotocin A, roflamycoin and dermostatin, it is seen that HA-1-92 is different from the earlier reported oxohexaenes. Therefore, on the basis of spectral (UV, IR, NMR, CIMS) data as well as elemental analysis of HA-1-92, and their comparison with the published data of reported oxohexaenes the chemical structure could be proposed as shown in Figure 2. It is also observed that HA-1-92 is a different from the other known oxohexaene (Figures 3-5).
Physicochemical data

<table>
<thead>
<tr>
<th>Oxohexaenes</th>
<th>Dermostatin</th>
<th>Mycoticin A</th>
<th>Roflamycoin</th>
<th>HA-1-92</th>
</tr>
</thead>
<tbody>
<tr>
<td>m.p. (Decomposed)</td>
<td>222-23°C</td>
<td>210°C</td>
<td>161-63°C</td>
<td>215-17°C</td>
</tr>
<tr>
<td>Optical Rotation</td>
<td>-82.0 (methanol)</td>
<td>+63.4 (dioxane)</td>
<td>-45.0 (dioxane)</td>
<td>-89.29 (methanol)</td>
</tr>
<tr>
<td>TLC (Rt value)</td>
<td>0.78</td>
<td>0.85</td>
<td>0.88</td>
<td>0.323</td>
</tr>
<tr>
<td>(BuOH : CH₃COOH :H₂O)</td>
<td>(4: 1 : 5)</td>
<td>(4: 1 : 5)</td>
<td>(4: 1 : 5)</td>
<td>0.52</td>
</tr>
<tr>
<td>HPLC*</td>
<td>2.5 &amp; 2.9</td>
<td>Sample not available</td>
<td>Sample not available</td>
<td>3.23, 3.8, 4.81, 5.62</td>
</tr>
<tr>
<td>(Retention time, min)</td>
<td>(major peak 2.5)</td>
<td>Sample not available</td>
<td>Sample not available</td>
<td>&amp; 6.47 (Major peak 3.23)</td>
</tr>
<tr>
<td>Mol formula (Mol. wt)</td>
<td>C₄₀H₄₄O₁₁ (720)</td>
<td>C₃₆H₄₈O₁₂ (650)</td>
<td>C₄₀H₄₆O₁₂ (738)</td>
<td>C₃₆H₄₄O₉ (560)</td>
</tr>
<tr>
<td>Toxicity (LD₅₀ i.p. route)</td>
<td>50 mg/kg</td>
<td>15 mg/kg</td>
<td>24 mg/kg</td>
<td>90 mg/kg</td>
</tr>
</tbody>
</table>

*Solvent system 0.05M sodium citrate buffer, pH 5.3: Acetonitrile (60 : 40), column C-18 (25 x 0.4 cm), flow rate (1ml/min) detection 380 nm (Hewlett Packard Model 8810)

Experimental Section

All organic solvents were duly purified and dried prior to their use. The crude powder of HA-1-92 obtained after extracting with butanol was concentrated to 1/10th of the volume by rotary vacuum evaporation and subsequently washed with different solvents and dried under vacuum at 40°C. The crude powder of HA-1-92 (1g) was dissolved in CH₃OH and purified by silica gel column chromatography by gradient elution with CH₃OH-C₆H₆ (1:9 and 9:1). Biologically active fractions tested for antifungal activity against (C. albicans, in vitro) were pooled and concentrated in vaccuo at 40°C to get brownish thick concentrate (0.709 g). Pooled active fractions from column chromatography were then subjected to HPLC (Hewlett Packard 8810 with C-18 column (0.4 x25cm, 10μ)). Mobile phase [CH₃CN – 0.05M sodium citrate buffer (40 : 60 v/v)] pH 5.3 was used with flow rate of 1 mL/min and detection was made by UV absorption at 3.5 nm. Pooled active fractions from column chromatography were then subjected to TLC (butanol-acetic acid – water ; 4 : 1 : 5 v/v) on silica gel plate (20 x 20cm), GF₂₅₄ (E. Merck). Major component was separated by extracting the major silica gel band with CH₃OH to get more pure HA-1-92 (0.58g). The purity and homogeneity of HA-1-92 was also confirmed by TLC employing different solvent systems and monitoring antifungal activity. The major component of HA-1-92 purified by TLC was also matched by HPLC (3.23 min retention time).
The purified HA-1-92 was used for various physico-chemical and spectral analysis, viz. solubility, specific rotation, pH, melting point, UV absorption and FTIR spectrum. Specific rotation was determined on Jasco DIP-181 digital polarimeter, melting point was determined on Thermonik (Campbell Electronics, Mumbai) electronically heated oil-bath and is uncorrected. UV measurements were made on UV-240 Shimadzu Spectrophotometer and FTIR spectrum was recorded on 8101 Shimadzu Infrared Spectrophotometer.

The $^1$H NMR (200 MHz) and $^{13}$C NMR (50 MHz) spectra were recorded on Bruker AC-200 using CD$_3$OD and CHCl$_3$ as solvents, respectively. Mass spectra were recorded on chemical ionization mode (CIMS) on a Finnigan MAT 1020 (RRL Jorhat, Assam) at 700 eV ionising beam using solid probe. Elemental analysis for C, H and N were performed at NCL, Pune.

Acknowledgement

Authors gratefully acknowledge the help rendered by Dr T. Ravindranathan, Dy Director, Organic Chemistry Division and his team at NCL, Pune and Dr K R Pillai, National Institute of Oceanographic Research Centre, Kerala. Authors are also grateful to the Director, CDRI, Lucknow, for supplying lyophil of CDRIL-312 and to the Director, RRL, Jorhat, for mass spectral analysis.

References