Advances in Contemporary Research

Natural camptothecins

Biswanath Das*, P Madhusudhan, P Veena Reddy & Y Anitha
Organic Chemistry Division-I, Indian Institute of Chemical Technology, Hyderabad 500 007, India

Camptothecin, a novel pyrrolo[3,4-b]-quinoline alkaloid, is a lead anticancer agent of the recent years. The compound and its several analogues (collectively known as camptothecins) have been reported from different botanical species. The former is also a promising drug in the AIDS chemotherapy. Several other camptothecins also exhibit significant antitumour and anti-HIV properties. The literature concerning the chemistry and bioactivity of these natural camptothecins has been briefly reviewed.

Investigation on natural anticancer agents is an important subject of the current research all over the world. Cancer is a growing public health menace and has been a major killer in most developed, developing and underdeveloped countries. As a result of the ceaseless efforts of different scientists certain ‘lead’ molecules have been discovered as Nature’s boon for cancer chemotherapy. One of the most impressive anticancer molecules of the recent years is camptothecin 1 which was originally isolated from a rare Chinese plant, *Camptotheca acuminata* Decne (Nyssaceae) under the natural anticancer agents screening programme carried out by the National Cancer Institute, USA. Indian *Notlapodytes foetida* (Wight) Sleumer (formerly, *Mappia foetida* Miers (Icacinaceae) has been found 2 to be an important source of the compound.

Camptothecin 1 is chemically a novel pyrrolo[3,4-b]-quinoline alkaloid. The interesting feature of the compound is the presence of a hydroxylactone ring in the molecule. 1 The compound shows remarkable antitumour and antileukaemic activities. 1 It is also a potential in the AIDS chemotherapy. 3 Several analogues of camptothecin (collectively known as camptothecins) have been reported from various botanical species (Table I). Some of these compounds also possess potent antitumour and anti-HIV properties. In the present article the literature reporting the chemistry and bioactivity of natural camptothecins is briefly reviewed.

Camptothecin 1

Camptothecin 1 has been isolated as a pale yellow compound and its structure was established from its spectral data, chemical modifications and X-ray crystallographic analysis. 1 Camptothecin does not form stable salts with acids. The presence of a hydroxyl group in the compound was evident from the formation of an acetate and a chloroacetate. The iodoacetate was prepared from the chloroacetate by using sodium iodide in acetone. Reaction of 1 with thionyl chloride and pyridine in benzene yielded chlorocamptothecin. Exhaustive hydrogenation of the former in acetic acid with Adams catalyst gave its dodecahydro derivative 1a.

The X-ray analysis of camptothecin was conducted on its iodoacetate (1b). 1 Crystals of camptothecin iodoacetate are orthorhombic having space group of P21 P21 P2, with four molecules of C22H17IN2O5 in a cell of dimensions of a=12.77, b=22.70, c=7.07 Å. X-ray intensity data were collected by means of equiinclination Weissenberg photographs. The absolute configuration was determined by Bijvoet’s method 49. It was based on the anomalous dispersion by the iodine atom of the CuK radiation.

Biological activity

Camptothecin 1 is well known for its potent antileukaemic and antitumour properties. 1,30-32 The compound tested against leukemia L1210 in mice gave life prolongation as high as 100% on a daily dose of 0.25-1.0 mg/kg and against Walker 256 (intramuscular) tumour (rats) with concentration as low as 1.25 mg/kg gave significant inhibition of growth. It also showed potent antitumour activity against P388 cells and B16 melanocarcinoma and moderate cytotoxi-
city against KB cell culture with ED50 of 0.07 μg/ml. Camptothecin is also useful for treatment of liver carcinoma and tumours of the head and neck. The compound possesses an unique mechanism of action.

Camptothecin also inhibits retroviruses such as the human immunodeficiency virus and the equine infectious anemia virus. The anti-HIV activity of the compound is due to the inhibition of Tat-mediated transcription from the viral promoter. Recently it has shown promising results against parasitic trypanosomes and leishmanias. However the major problem associated with the compound is its high toxicity and poor solubility.

Chemical modifications of camptothecin afforded several analogues. Two semisynthetic analogues, irinotecan (1c) and topotecan (1d) have been introduced in the clinic. Irinotecan showed broad spectrum of activity and it was approved in Japan for treatment of lung, cervical (ovarian) and breast cancers.

### 10-Hydroxycamptothecin 2

10-Hydroxycamptothecin, a minor alkaloid, is a monohydroxy derivative of 1 as it formed mono (2a) and diacetate (2b) compounds. The UV spectra of the acetates were similar to that of 1 thereby suggesting the presence of the same camptothecin chromophore in compound 2. The position of hydroxyl functional group was ascertained from the analysis of its 1H NMR spectral data. This assignment was further supported by the examination of the 1H NMR spectra of deuterated hydroxycamptothecin and of some related tricyclic synthetic model compounds. The structure received further confirmation from its 13C-NMR spectrum.

#### Biological activity

10-Hydroxycamptothecin 2 has been found to be more active and more potent in P-388 mouse leukemia than camptothecin 1. It is used clinically in the People's Republic of China for the treatment of stomach and liver cancers.

### 10-Methoxycamptothecin 3

The structure of 10-methoxycamptothecin was settled by comparison of its spectral data with those of synthetic 10-methoxycamptothecin prepared from 10-hydroxycamptothecin by methylation with diazomethane.

#### Biological activity

10-Methoxycamptothecin has not initially been reported as an inhibitor of herpes virus. It has been shown to fragment DNA. However, in a later study it was observed that the compound was about eight times more potent than camptothecin as an inhibitor of herpes virus as measured by plaque reduction. At levels of 20 and 10 μg/mL of nutrient-agar overlay, 10-methoxycamptothecin gave 100% and 89% inhibition of plaques while no gross toxicity was observed in preformed monolayers at levels as high as 100 μg/mL.

### 9-Methoxycamptothecin 4

Besides the plant sources mentioned in Table I the cell and tissue culture of Notihepatoidea foetida and Camptotheca acuminata also afforded 9-methoxycamptothecin in low yields.

The structure of the 9-methoxycamptothecin has been derived from the spectral data of the compound.
and of its acetyl derivative. The C.D. (in dioxane) of the alkaloid was virtually superposable with that of camptothecin and showed that they have the same absolute configuration (S) at the sole asymmetric centre at C-20.

**Biological activity**

9-Methoxycamptothecin has shown significant cytotoxic activity against human epidermoid carcinoma, (ED50 μg/mL): KB cells (1.8×10^-4), P-388 (9.9×10^-5), A-549 (3.6×10^-5), HT-69 (3.0×10^-5) and HL-60 (1.5×10^-5). The compound has also shown substantial activity in the B16 melanoma, L-1210 lymphoid leukemia, Lewis Lung carcinoma and P-388 lymphocytic leukemia test systems.

**Mappicine 5**

Mappicine lacks the δ-lactone (1R: 1745 cm^-1) present in camptothecin. The most significant differences between the ^1^HNMR spectra of mappicine and camptothecin concern with the groups present in ring E of camptothecin. Mappicine shows the presence of an aromatic methyl and hydroxy propyl group. The triplet at δ5.14 (CHOH) of mappicine is shifted to δ5.90 in the acetate indicating the structure of the former to be 5. The O.R.D. and C.D of mappicine showed negative cotton effect in the 300-400 nm suggesting the S-configuration at C-20.

The synthesis of recemic mappicine was achieved by reduction of camptothecin with NaBH₄ followed by treatment with lead tetraacetate and subsequent reduction of the product with NaBH₄.

The stereoselective synthesis of mappicine 5 has also recently been carried out from camptothecin. The latter was irradiated under microwave irradiation to give mappicine ketone 25 which on treatment with baker’s yeast afforded natural mappicine with high optical purity.
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name (structure)</th>
<th>Mol. formula</th>
<th>m.p. °C (solvent)</th>
<th>[α]D (solvent)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Camptothecin 1</td>
<td>C20H16N2O4</td>
<td>270-271 (glacial HOAc)</td>
<td>+42.5° (CHCl₃-MeOH, 4:1)</td>
<td>Camptotheca acuminata Decne (Nyssaceae)⁴¹,⁴²,⁴³,⁴⁴ Nathapodytes foetida (Weight) Sleumer (formerly, Mappia foetida Miers) (Icacinae)³⁵,³⁶ Ophiiorrhiza nunglos Linn (Rubiaceae)⁷⁹ Ervatamia heyneana (Wall) T. Cooke (Apocynaceae)³⁰ Merrilliodendron megacarpum (Hmsl.) Sleum (Icacinae)³³ Ophiiorrhiza pulula Champ. (Rubiaceae)³²,³⁶ Mostuea brunonis Didr. (Loganiaceae)³⁷</td>
</tr>
<tr>
<td>2</td>
<td>10-Hydroxycamptothecin 2</td>
<td>C20H16N2O3</td>
<td>268-270 (EtOAc-MeOH-CHCl₃)</td>
<td>—</td>
<td>Camptotheca acuminata⁷,⁹,¹¹,¹³,¹⁴,¹⁷ Nathapodytes foetida¹⁷</td>
</tr>
<tr>
<td>3</td>
<td>10-Methoxycamptothecin 3</td>
<td>C21H18N2O5</td>
<td>254-255 (EtOAc-MeOH-CHCl₃)</td>
<td>—</td>
<td>Camptotheca acuminata⁹,¹¹,¹³,¹⁴,¹⁵,¹⁷ Ophiiorrhiza nunglos⁷⁹</td>
</tr>
<tr>
<td>4</td>
<td>9-Methoxycamptothecin 4</td>
<td>C21H18N2O5</td>
<td>258-259 (d) (CHCl₃-MeOH)</td>
<td>-77.2° (C₅H₅N)</td>
<td>Camptotheca acuminata⁷,¹¹,¹³,¹⁴,¹⁵,¹⁷ Ophiiorrhiza nunglos⁷⁹ Nathapodytes foetida²,¹⁵,²⁴,²⁶,²⁸,³⁰,³¹</td>
</tr>
<tr>
<td>5</td>
<td>Mappicine 5</td>
<td>C20H16N2O2</td>
<td>251-252</td>
<td>-12.4° (CHCl₃- MeOH, 1:1)</td>
<td>Camptotheca acuminata⁷,⁹</td>
</tr>
<tr>
<td>6</td>
<td>20-Deoxycamptothecin 6</td>
<td>C20H16N2O2</td>
<td>0° (CHCl₃)</td>
<td>—</td>
<td>Camptotheca acuminata⁷,¹¹,¹³,¹⁴,¹⁵,¹⁷</td>
</tr>
<tr>
<td>7</td>
<td>11-Hexanoylcamptothecin 7</td>
<td>C20H16N2O2</td>
<td>326-327</td>
<td>30.7° (C₅H₅N)</td>
<td>Camptotheca acuminata⁷,¹¹,¹³,¹⁴,¹⁵,¹⁷</td>
</tr>
<tr>
<td>8</td>
<td>20-Hexanoylcamptothecin 8</td>
<td>C20H16N2O3</td>
<td>238-242</td>
<td>-26° (CHCl₃)</td>
<td>Camptotheca acuminata⁹</td>
</tr>
<tr>
<td>9</td>
<td>20-Hexanoyl-10-methoxy camptothecin 9</td>
<td>C20H16N2O3</td>
<td>238-242</td>
<td>-26° (CHCl₃)</td>
<td>Camptotheca acuminata⁹</td>
</tr>
<tr>
<td>10</td>
<td>18-Hydroxycamptothecin 10</td>
<td>C20H16N2O3</td>
<td>256-258</td>
<td>-21.40 (C₅H₅N)</td>
<td>Camptotheca acuminata¹¹,¹⁵</td>
</tr>
</tbody>
</table>

---Contd.
### Table I—Naturally occurring camptothecins

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name (structure)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>18,19-Dehydrocamptothecin <strong>11</strong></td>
<td><em>Nothapodytes foetida</em>[^17^]</td>
</tr>
<tr>
<td>12</td>
<td>10-Hydroxydeoxycamptothecin <strong>12</strong></td>
<td><em>Camptotheca acuminata</em>[^10^]</td>
</tr>
<tr>
<td>13</td>
<td>19-Hydroxy mappicine <strong>13</strong></td>
<td><em>Camptotheca acuminata</em>[^46^]</td>
</tr>
<tr>
<td>14</td>
<td>Pumiloside (<strong>14</strong>)</td>
<td><em>Ophiolirhiza punila</em>[^32^,^34^,^36^]</td>
</tr>
<tr>
<td>15</td>
<td>3(R)-Deoxypumiloside (<strong>15</strong>)</td>
<td><em>Camptotheca acuminata</em>[^11^]</td>
</tr>
<tr>
<td>16</td>
<td>22-Hydroxycumatinine (<strong>16</strong>)</td>
<td><em>Camptotheca acuminata</em>[^46^]</td>
</tr>
<tr>
<td>17</td>
<td>Chaboside (<strong>17</strong>)</td>
<td><em>Ophiolirhiza punila</em>[^33^,^34^]</td>
</tr>
<tr>
<td>18</td>
<td>20-O-Acetyljcamptothecin (<strong>18</strong>)</td>
<td><em>Nothapodytes foetida</em>[^20^,^23^]</td>
</tr>
<tr>
<td>19</td>
<td>Mappicine 20-O-β-D-glucopyranoside</td>
<td><em>Nothapodytes foetida</em>[^19^]</td>
</tr>
<tr>
<td>20</td>
<td>Mappicine 20-O-β-D-gentiobioside</td>
<td><em>Nothapodytes foetida</em>[^19^]</td>
</tr>
<tr>
<td>21</td>
<td>17-Hydroxymappicine-20-O-β-D-glucopyranoside</td>
<td><em>Nothapodytes foetida</em>[^19^]</td>
</tr>
<tr>
<td>22</td>
<td>9-Methoxymappicine 20-O-β-D-gentiobioside</td>
<td><em>Nothapodytes foetida</em>[^19^]</td>
</tr>
<tr>
<td>23</td>
<td>Foetidin (<strong>23</strong>)</td>
<td><em>Nothapodytes foetida</em>[^19^]</td>
</tr>
</tbody>
</table>

[^17^]: Dehydrocamptothecin is isolated from *Nothapodytes foetida*.
[^10^]: Hydroxydeoxycamptothecin is isolated from *Camptotheca acuminata*.
[^46^]: Hydroxy mappicine is isolated from *Camptotheca acuminata*.
[^32^]: Pumiloside is isolated from *Ophiolirhiza punila*.
[^34^]: Pumiloside is isolated from *Camptotheca acuminata*.
[^36^]: Pumiloside is isolated from *Nothapodytes foetida*.
[^11^]: 3(R)-Deoxypumiloside is isolated from *Camptotheca acuminata*.
[^33^]: Chaboside is isolated from *Ophiolirhiza punila*.
[^34^]: Chaboside is isolated from *Ophiolirhiza punila*.
[^20^]: 20-O-Acetylcamptothecin is isolated from *Nothapodytes foetida*.
[^23^]: 20-O-Acetylcamptothecin is isolated from *Nothapodytes foetida*.
[^19^]: Mappicine is isolated from *Nothapodytes foetida*.
[^19^]: Mappicine is isolated from *Nothapodytes foetida*.
[^19^]: 17-Hydroxymappicine is isolated from *Nothapodytes foetida*.
[^19^]: 9-Methoxymappicine is isolated from *Nothapodytes foetida*.
[^19^]: Foetidin is isolated from *Nothapodytes foetida*.

---Contd
Table I—Naturally occurring camptothecins

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name (structure)</th>
<th>Mol. formula</th>
<th>m.p. °C (solvent)</th>
<th>[α]D (solvent)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>(9-Methoxy mappicine ketone)</td>
<td>C20 H28 N2 O5</td>
<td>235-238 (CHCl3)</td>
<td>0°</td>
<td>Nathapodytes foetida^{23,26,37}</td>
</tr>
<tr>
<td>25</td>
<td>(Mappicine ketone)</td>
<td>C18 H16N2 O4</td>
<td>210-215 (CHCl3)</td>
<td>0°</td>
<td>Nathapodytes foetida^{23,26,37}</td>
</tr>
<tr>
<td>26</td>
<td>(S)-Deoxypumiloside</td>
<td>C30 H42 N2 O12</td>
<td>20-0-D-Glucopyranosyl</td>
<td>-23.5° (MeOH)</td>
<td>Ophiorrhiza pumila^{35}</td>
</tr>
<tr>
<td>27</td>
<td>9-β-D-Glucosylcamptothecin</td>
<td>C26 H36 N2 O10</td>
<td>9-β-D-Glucosyloxycamptothecin</td>
<td>C26 H36 N2 O10</td>
<td>Ophiorrhiza pumila^{34,38}</td>
</tr>
<tr>
<td>28</td>
<td>20-O-β-D-Glucopyranosyl camptothecin</td>
<td>C32 H46 N2 O12</td>
<td>20-0-β-D-Glucopyranosyl camptothecin</td>
<td>C32 H46 N2 O12</td>
<td>Mostuca brunonis^{37}</td>
</tr>
<tr>
<td>29</td>
<td>9-Methoxy mappicine</td>
<td>C20 H26 N2 O5</td>
<td>249-251 (MeOH)</td>
<td>-9.65° (CHCl3-MeOH, 4:1)</td>
<td>Nathapodytes foetida^{28}</td>
</tr>
</tbody>
</table>

The compounds are listed according to the chronological order of their first isolation.

20-Deoxycamptothecin 6

The compound^{39} was identical chromatographically and spectroscopically with the synthetically prepared racemic 20-deoxycamptothecin from camptothecin. The isolated natural compound was oxidized to camptothecin by the method of Winterfeldt.^{64}

11-Hydroxycamptothecin 7

The accurate mass determination suggested^{44} the compound to be a position isomer of hydroxycamptothecin. Direct comparison of this compound with authentic 9-hydroxycamptothecin eliminated the 9-position. Position 11 for the OH group was confirmed by direct comparison with demethylated product of racemic 11-methoxycamptothecin (7a).^{44}

Biological activity

Racemic 11-hydroxycamptothecin (as the natural 20 S compound was obtained in trace quantity) was tested for activity in the L1210 mouse leukemia assay.^{34} The compound exhibited much greater activity than camptothecin, with 3 out of 6 cures and a very high % of T/C (357) (based on survival time). The racemic compound also exhibited a greater therapeutic index than camptothecin. It should be noted that racemic camptothecin was about half as potent as (20 S)-camptothecin. Hence it is likely that natural (20 S)-11-hydroxycamptothecin would be considerably more potent than racemic 11-hydroxycamptothecin.

20-Hexanoylcamptothecin 8

Spectroscopically (IR and 1H NMR) this compound was identical to synthetically prepared 20-hexanoylcamptothecin from camptothecin.^{9}

20-Hexanoyl-10-methoxycamptothecin 9

The structural assignment of the compound was correlated from its 1H NMR and MS spectral data with those of camptothecin.^{9}

18-Hydroxycamptothecin 10

The structure of the compound was settled^{45} by spectroscopic analysis. Its stereochemistry at C-20 was established as S from its C.D. spectra.

Biological activity

18-Hydroxycamptothecin (10) showed a strong cyto-

---

^{1}The compounds are listed according to the chronological order of their first isolation.
toxicity against P-388 leukemia cells in vitro. 45

18,19-Dehydrocamptothecin 11
HRMS of the compound showed that it differs by 2 mass units from that of camptothecin.17 In its 1H NMR spectrum, typical ABC spin coupling pattern attributed to a vinyl group appeared at 85.39 (1H, d, J=10.5 Hz), 5.40 (1H, d, J=17.6 Hz) and 85.89 (1H, dd, J=10.3 Hz and J=17.6 Hz) instead of ethyl group of camptothecin. Hydrogenation of 11 in the presence of palladium catalyst gave camptothecin quantitatively.

The C.D. spectrum of 18,19-dehydrocamptothecin 11 showed a positive cotton effect [(θ)241 + 24400] and agreed with that of camptothecin (1) [(θ)238 + 59400]. The hydrogenation product also showed similar C.D. spectrum to that of camptothecin (1). The stereochemistry of 11 was thus established as S.

Camptothecin was said to be biosynthesized from tryptamine, derived from tryptophan, and iridoid secologonin in plants.53 The vinyl group in 18,19-dehydrocamptothecin 11 seemed to originate from that of secologonin.

10-Hydroxydeoxycamptothecin 12
The structure of the compound was established10 with 20R-configuration from its spectral data and chemical transformations.

Biological activity
10-Hydroxydeoxycamptothecin 12 showed prominent cytotoxicity against P-388 leukemia.

19-Hydroxymappicine 13
The compound was obtained as yellow crystals.46 The UV spectrum of this alkaloid 13 is similar to that of camptothecin 1. The 1H NMR spectral properties of 13 were identical to that of mappicine 5, except that the former had one more hydroxyl group in the side chain. The position of the hydroxyl group was settled as C-19 by 1H NMR and NOE spectral studies. Like mappicine, this compound also showed negative Cotton effects in the region 300-400 nm suggesting S-configuration at C-20.

Pumiloside 14
Pumiloside was postulated as the poststrictosamide intermediate53 of camptothecin biosynthesis. The UV spectrum of the compound indicated the presence of quinoline chromophore in the molecule. The structure was settled by extensive studies of its 1H NMR and 13C NMR spectra and was finally confirmed from X-ray diffraction analysis. The synthesis of pumiloside was carried out from tryptamine and secologonin for clarifying the stereostructure. The synthetically prepared pumiloside was fully identical to natural pumiloside. The synthesis of C-3 epimer of pumiloside has also been made to obtain the unambiguous proof of the stereostructure. This synthesis clearly established the configuration at C-3 as also the configurations at C-15, 20 and 21. The C.D. spectra of pumiloside and 3-epipumiloside showed the opposite sign of the cotton effect of the longest wavelength region (350-300 nm), positive for 14 and negative for its C-3 epimer. It clearly verified the assigned stereochemistry.

3(R)-Deoxypumiloside 15
The molecular formula of the compound corresponds to a deoxy analogue of pumiloside (14).32,36 The structure of the compound was suggested as 7-deoxypumiloside 15 from its UV and 1H NMR spectra. The α-H-3 configuration was indicated by the C.D. spectrum possessing a curve similar to pumilo-
side 14. However, synthesis of the compound from vicinose lactam suggested\textsuperscript{3} \textsuperscript{3}(R)-configuration with β-H. This deoxypseudomside 15 may be another member of the long-sought poststric-tosamide intermediate of camptothecin biosynthesis.\textsuperscript{33}

**22-Hydroxycuminatine 16**

The structure of the compound was established by spectral analysis.\textsuperscript{46} The \textsuperscript{1}H NMR spectrum suggested that A, B, C and D rings are identical to those of camptothecin. Remaining three aromatic protons and one hydroxy methyl group should be located on the E ring which should be fully aromatic because those three protons were coupled each other. The hydroxy methyl group was reasonably placed at C-16 from the study of the NOE and COSY spectra of the compound.

22-Hydroxycuminatine 16 is a biogenetically novel alkaloid as its A-D rings are similar to those of camptothecin while the E-ring is of the yohimbane type.

**Biological activity**

22-Hydroxycuminatine 16 showed\textsuperscript{46} cytotoxic activity against the P388 and KB test systems in vitro with ED50 values of 1.32 and 0.61 μg/ml respectively.

**Chaboside 17**

The UV spectrum of compound showing a series of complex absorption bands in the region between 222 and 380 nm was characteristic of camptothecinoids.\textsuperscript{33,34} Compound 17 was shown as a hexoside of a methoxyhydroxy camptothecin. The NMR spectra of the compound showed that its B, C, D and E rings are similar to those of camptothecin. The characteristic feature of the structure of 17 was on its A ring. The signals due to one aromatic methoxy group (\textsuperscript{1}H-NMR: 4.09, \textsuperscript{13}C-NMR: 62.5) and the protons and carbons of glucose moiety were easily recognized. Since two aromatic protons were observed to couple each other with J value of ortho-coupling (J=9.35 Hz), the methoxy and glucosyloxy groups can be located either at 9 and 10, or 11 and 12 on the A ring. The exact position of these substituents was determined from the NOESY and COLOC spectra. The NOESY spectrum demonstrated distinct NOE cross peaks between the methoxy protons (δ 4.09) and B ring proton H-7 (δ 8.7), and also between the anemic proton H-1 (δ5.18) and the aromatic proton H-11 (δ7.72). From these observations positions C-9 for methoxy and C-10 for glucosyloxy groups were deduced. Furthermore this assignment was verified by COLOC spectra. All other NOESY and COLOC signals supported the structure 17 for chaboside. The latter is the first natural glucoside of a true camptothecinoid. It is also the first-natural camptothecin carrying two oxygen functions on the A ring.

**20-O-Acetylcamptothecin 18**

The UV spectrum of 20-O-acetylcamptothecin was very close to that of camptothecin.\textsuperscript{20,21} The \textsuperscript{1}H NMR spectrum of the compound differs from that of 1 only by the presence of a signal at δ 2.22 (3H, s) for acetyl group instead of a signal at δ 6.50 for hydroxyl in 1. This was further supported by the IR spectrum at 1745 cm\textsuperscript{-1} for an ester carbonyl group. The position of the acetyl group at C-20 was evident from \textsuperscript{1}H NMR spectral data and acetylation of camptothecin (1). Both the natural and synthetic 20-O-acetylcamptothecin showed identical physical and spectral properties indicating the 20(S)-configuration of the compound.

**Biological activity**

20-O-Acetylcamptothecin showed significant cytotoxic activity in the KB, P-388, A-549, HT-29 and HL-60 tissue culture assays.\textsuperscript{29}

**Mappicine 20-O-β-D-glucopyranoside 19**

The compound showed in the \textsuperscript{1}H NMR spectrum all the resonances and multiplicities associated with the A,B,C and D rings of camptothecin.\textsuperscript{19} The verification of the \textsuperscript{1}H- and \textsuperscript{13}C-NMR spectra also indicated the absence of the AB system at δ5.44 and the lactone carbonyl (C-21) at δ73.45 as well as the presence of a singlet at δ12.16 (3H, H-17) and a triplet at δ5.14 (1H, J=6.7 Hz, H-20). These data suggested that the compound 19 lacked the E ring of camptothecin. The FAB-MS spectrum of 19 showed a pseudomolecular peak at m/z 469 (M+H)+, which corresponded to the molecular formula C\textsubscript{25}H\textsubscript{28}N\textsubscript{2}O\textsubscript{7}, and a fragment at m/z 307 (M-162+H)+. Moreover both in the \textsuperscript{1}H and \textsuperscript{13}C NMR spectra, the signals of a O-β-D-glucosyl residue were readily identified. This moiety could only be linked at the C-20 position. From all the spectroscopic data compound 19 was thus assigned the structure of mappicine 20-O-β-D-glucopyranoside. The C.D. spectrum showed a negative cotton effect in the region 300-400 nm which suggested the (S)-configuration at C-20.
Mappicine 20-\(\text{O-}\beta-\text{d-gentiobioside 20}\)

The \(^1\)H and \(^13\)C-NMR spectra of the compound confirmed the mappicine skeleton as well as the presence of a gentiobiosyl moiety at C-20.\(^{19}\) Thus the compound was assigned the structure of mappicine 20-\(\text{O-}\beta-\text{D-gentiobioside 20}\). The C.D. spectrum showed a negative cotton effect in the region 300-400 nm suggesting the (S)-configuration at C-20.

17-Hydroxymappicine-20-\(\text{O-}\beta-\text{d-glucopyranoside 21}\)

The \(^1\)H and \(^13\)C-NMR spectra of the compound showed similar protons signals for the A, B, C and D rings of camptothecin.\(^{19}\) The singlet at \(\delta 2.16\) of 1 was substituted by an AB system in 19 at \(\delta 4.65\) and \(\delta 4.49\) (d, \(J=11.4\) Hz) which suggested the presence of an alcohol group at C-17. A glucosyl moiety was placed at C-20 from the examination of its \(^1\)H and \(^13\)C resonances. The C.D. spectrum showed negative cotton effect in the region 300-400 nm which suggested the (S)-configuration at C-20.

17-Hydroxymappicine itself was not isolated from a natural source but only obtained\(^{13}\) as a racemate during the partial synthesis of mappicine (5) from camptothecin.

9-Methoxymappicine-20-\(\text{O-}\beta-\text{d-gentiobioside 22}\)

The \(^1\)H NMR spectrum of the compound showed peculiar differences in the aromatic region in comparison with the previous isolated compounds 19-21 from the same source.\(^{19}\) Five resonances in the aromatic region were readily identified instead of the usual six protons present in compounds 19-21 and camptothecin. Moreover, a singlet at \(\delta 4.01\) (3H) suggested the presence of an arylmethoxy group in 22. The \(^1\)H NMR spectrum of the latter showed the 9-methoxy substitution in the A ring and the A, B, C and D rings were similar to those of 9-methoxy camptothecin (4). The signals of the other aromatic and aliphatic protons were identical to those reported for the compound 20. Similar assignments were also made from the \(^13\)C NMR spectrum. The compound was identified as 9-methoxymappicine-20-\(\text{O-}\beta-\text{D-gentiobioside. From the C.D. spectrum (S)-configuration at C-20 of the compound was determined.}\)

Foetidin I 23

The \(^1\)H NMR spectrum of the compound was more complex in comparison with the spectra of compounds 19-22.\(^{15}\) The spectrum showed that the A, B, C and D rings were identical to those of camptothecin (1). The two protons at C-17 are bonded to one acentoxy group as shown by the long-range correlation between H-17 and the acetate carbonyl. The ethyl moiety (C-18, C-19) was present, bonded to an oxygenated carbon (C-20), as in 1. The two \(\text{D-O-}\) exchangeable protons present at low field (\(\delta 8.19\) and 8.04, 1) were due to two secondary amide groups. Two almost equivalent para-substituted benzene rings were also recognized (\(\delta 6.76\) (4H, d), 7.34 (2H, d) and 7.35 (2H, d)). For both benzene rings one substituent was -\(\text{CH=CHCO-}\) moiety (57.29 (d), 6.36 (d), 6.35 (d) and 7.28 (d)), while the second was either a phenolic-OH or its derivative. The \(^13\)C-NMR spectrum showed seven methylene carbons, two bonded to an amine nitrogen (\(\delta 47.23\) and 45.36), two bonded to an amide nitrogen (\(\delta 38.41\) and 36.36) and three were aliphatic (626.29, 26.87 and 24.04). Simple spin-spin decoupling experiments assign the structure of spermidine to the nitrogen containing aliphatic fragment present in compound 23. Both methylene groups at \(\delta 3.14\) and 3.20 (H-11 and H-18) showed a long-range coupling with the two carbonyls at \(\delta 165.84\) (C-9') and 165.27 (C-20') respectively, thus demonstrating that the first and the last nitrogen of spermidine (N-10' and N-19') were bonded through an amide function to the two coumaric acid moieties. The mass spectrum (FABMS) for compound 23 showed a peak at \(m/z\) 438, which corresponded to the fragment [C\(_{25}\)H\(_{23}\)N\(_2\)O\(_{10}\)]\(^+\) and supported the presence of a di-p-coumaroylspermidine moiety. The other peaks at \(m/z\) 349 [C\(_{25}\)H\(_{37}\)N\(_2\)O\(_{14}\)]\(^+\) and 305 (349-CO\(_2\))\(^-\) were characteristic of camptothecin.

The chemical conversion of foetidin suggested the C-20 hydroxyl as the site for binding the side chain through an ester bond. When the compound was left at room temperature for 72 hr in a buffered solution (pH 4.5), it gave camptothecin almost quantitatively. Such behavior was more consistent with a phenol ester linkage than with phenol ether. All these spectral and chemical informations supported the structure 23 for foetidin I.
The C.D. spectrum of compound 23 showed a negative cotton effect in the region 300-400 nm. This suggested the (5)-configuration at C-20 of the compound.

**Biological activity**

In preliminary experiments on monolayer stabilized ovarian cells A2780 WT foetidin I (23) showed an IC_{50}=3.4×10^{-7} mol dm^{-3}, whereas camptothecin (1) on the same cell line had an IC_{50}=2.6×10^{-8} mol dm^{-3}. On herpes and HIV viruses, foetidin I showed potent antiviral activity (IC_{50}=0.6 µg cm^{-3}).

**Nothapodytine A (9-Methoxymappicine ketone)** (24)

The A-D rings of nothapodytine A (24) were found to be similar to those of 9-methoxycamptothecin 4 from its spectral data. The ^1H NMR spectrum of 24 differed from that of 4 only in the presence of a singlet signal at δ2.25 (3H) for an aromatic methyl group and the signals at δ1.24 (3H) and 2.90 (2H) for an 1-oxopropyl group in the former instead of the E ring in 4. The presence of the 1-oxopropyl group in 24 was also supported by the fragment ion at m/z 298 (M-C_3H_5O_2t). The arrangement of these two substituents at positions C-16 and C-15 respectively was suggested by the NOEs at δ5.80 (δ_H-3) and δ3.08 (δ_3-15) of the minor compound 26 led to an enhancement (4%) of the peak intensity of H-19 (δ 5.80) indicating that it has the 3(S)-configuration (δ_H-3).

The structures were confirmed by partial synthesis of the acetates of both deoxypumilosides using vincosidelactam and strictosamide as the starting materials respectively. The spectroscopic data of the synthetic 3(R) compound were identical with those of the acetate of deoxypumiloside which was earlier reported by Aimi et al. 3(5)-Deoxypumiloside tetraacetate was prepared from strictosamide (which possesses the a- H-3 configuration) via the reductive deoxygenation at C-7 of the intermediate 3(S)-pumiloside tetraacetate. Thus the absolute stereochemistry of both the deoxypumilosides was unambiguously established.

**9-β-D-Glucosylcamptothecin 27**

9-β-D-Glucosylcamptothecin (27) is a new hydroxyglucoside which was obtained from the regenerated plantlets of *Ophiiorhiza punila* from callus culture. The metabolite was isolated from acetylated BuOH extract and its structure was elucidated as 9-β-D-glucosylcamptothecin penta acetate (27a) based on spectrosopic studies. The UV spectrum of 27a is similar to that of 9-methoxycamptothecin (4). In the ^1H NMR spectrum a set of three aromatic protons due to the 1,2,3 trisubstituted benzene ring system in A ring, two singlet aromatic protons due to H-7(δ 8.68) and H-11 (δ 7.25), protons due to one ethyl group, one sugar unit and five acetyl groups and two methylene protons were observed. In the ^13C NMR spectrum the amide carbonyl (δ157.3) and lactone carbonyl (δ167.5) were apparent. The sugar part was determined as the β-linked glucose by both of the coupling con-
stant in the $^1$H NMR and chemical shifts in the $^{13}$C NMR spectra. In the HMBC spectrum cross peaks between the anomeric proton (δ 55.34) and C-9 (δ 152.2) and between H-7 (δ 78.68) in the ring B and C-9 (δ 152.2) were observed. The position of the glucosyl group at C-9 was concluded after an extensive NMR study. The absolute stereochemistry at C-20 was most probably (S) as is the case with other natural camptothecins (based on a C.D. spectral comparison). The validity of the proposed structure including the absolute configuration at the chiral center was unambiguously proved by the chiral total synthesis. The spectral data of synthetic compound was identical with the pentaacetate derived from the natural glucoside. Thus the structure of the compound 27a including the absolute configuration of the sugar moiety and the C-20 position was established. Deacetylation of 27a with $\text{K}_2\text{CO}_3$ (10 equiv.) in MeOH at room temperature gave 9-β-D-gluco-syloxycamptothecin (27) in 80% yield.

**20-O-β-Glucopyranosyl camptothecin 28**

The compound showed a pseudomolecular ion at m/z 511.1698 in HRFABMS corresponding to the molecular formula $\text{C}_{30}\text{H}_{30}\text{N}_2\text{O}_8$ and a fragment ion at m/z 349 in LRCIMS corresponding to the $[\text{M-glucose}]^{+}$ ion. The $^1$H- and $^{13}$C-NMR spectra indicated the presence of a glucosyl unit as well as the camptothecin nucleus in 28. The β-configuration of the anomeric position (C-1') of the sugar was considered from the coupling constant between H-1' and H-2' (J = 7.5 Hz) and the $^{13}$C-NMR shift of C-1' (δ 101.1). The glucosyl unit was placed at C-20 on the basis of an HMBC correlation form the anomeric proton (H-1') at δ 4.67 to the C-20 signal at δ 79.1. The structure of 28 was thus established as 20-O-β-D-glucopyranosyl camptothecin.

**Biological activity**

20-O-β-D-Glucopyranosylcamptothecin showed a marginal response in the NCI 60 cell line. The compound has greater solubility in water and alcohol than camptothecin and can more readily be formulated.

**9-Methoxymappicine 29**

The $^1$H NMR spectrum of the compound is similar in the aromatic region to that of 9-methoxymappicine (4) and 9-methoxyxapicine ketone (24) while the spectrum of the non-aromatic region is identical to that of mappicine (5). On oxidation with PCC the compound afforded 24. This was further supported from its $^{13}$C NMR spectral data. The C.D. spectrum suggested its 20-(S) configuration.

An efficient chemoenzymatic synthesis of the compound starting from 9-methoxycamptothecin (4) has been achieved. The latter was irradiated under microwave irradiation to afford 9-methoxymappicine ketone (24) which was reduced with baker’s yeast at pH to produce the natural 9-methoxy-20(S)-mappicine (29).

**Acknowledgement**

The authors thank CSIR, New Delhi for financial assistance.

**References**

2. Govindachari T R & Viswanathan N, Phytochemistry, 11, 1972, 3529