Fast Atom Bombardment Mass Spectrometry of the Vitamin B<sub>12</sub> Analogues Hydrogenobalamin and Cupribalamin

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Hydrogenobalamin (metal-free vitamin B<sub>12</sub>) and cupribalamin are characterized by their fast atom bombardment (FAB) mass spectra which show molecular ion and characteristic fragment ion peaks. These spectra and the high-resolution FAB mass spectrum of cobalamin (vitamin B<sub>12</sub>) show that the (M+H<sup>+</sup>)–CN–59 peak for the latter is due to loss of acetamide and not of the central cobalt atom. In the FAB mass spectrum of cupribalamin metal clusters are observed.

Mass spectrometric investigations of biooligomers and of polar biomolecules can only be carried out with difficulty using the conventional ionization methods electron impact (EI), field desorption (FD) and chemical ionization (CI). On the other hand the new soft fast atom bombardment (FAB) ionization method [1] is ideally suited for the study of such compounds because it not only furnishes molecular mass information but also gives intense fragment ions [2], which are important for structure elucidation, and sequence ions in the case of oligomers [3].

Barber and coworkers [2] have shown the applicability of FAB mass spectrometry to cyanocobalamin (vitamin B<sub>12</sub>), to methyl- and hydroxocobalamin and to coenzyme B<sub>12</sub>, whereas Schwarz et al. [4] studied some (less polar) esterified derivatives of dicyanocobyrinic acid.

Here we wish to report positive and negative ion FAB mass spectra of hydrogenobalamin (metal-free vitamin B<sub>12</sub>) [5] and of cupribalamin [5] and we solve the ambiguity [2] concerning the loss of 59 mass units (Co or CH<sub>2</sub>CONH<sub>2</sub>) from the M+H–CN ion of cyanocobalamin by high-resolution positive ion FAB mass spectrometry.

Metal-free vitamin B<sub>12</sub> and its copper analogue are of interest because they have previously been shown to act as strong antimetabolites of vitamin B<sub>12</sub> in several bioassay systems [6, 7]. Furthermore, hydrogenobalamin is industrially used as starting material for the preparation of [<sup>57</sup>Co]-labelled vitamin B<sub>12</sub>.

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Fig. 1 shows the molecular ion regions of the positive and negative ion FAB mass spectra of hydrogenobalamin. In the positive ion spectrum a strong peak is found for the protonated molecular ion at 1272 d; the negative ion spectrum shows a molecular ion at 1270 d which is not completely deprotonated. The molecular ions are accompanied by oxygen-containing artefact ions.

Fig. 2 shows the molecular ion regions of the positive and negative ion FAB mass spectrum of cupribalamin. In the positive ion spectrum one ob-
Fig. 1. Molecular ion regions of the positive and negative ion FAB mass spectra of hydrogenobalamin.
serves an intense protonated molecular ion at 1333 d together with copper-containing cluster ions at 1395 d and 1457 d. The isotope composition of the cluster at 1395 d corresponds to an ion containing two copper atoms; the cluster at 1457 d contains three copper atoms. The negative ion spectrum displays both the deprotonated molecular ion peak at 1331 d and an intense molecular ion peak at 1332 d. The latter can be explained by reduction of copper from the cupric to the cuprous state. Analogous results were obtained by Barber in FAB studies of other organometallic compounds [8]. Metal clusters are only weakly visible in the negative ion spectrum and are attributed exclusively to the $M^-$ ion.

In Table I the main fragments postulated by Barber [2] for cyanocobalamin are correlated with
the fragments found by us in the positive ion FAB mass spectra of cupribalamin and hydrogenobalamin. The parallel fragmentation sequences indicate identical basic molecular structures. For cupribalamin and hydrogenobalamin one notices the same loss of 59 mass units from the M+H ions which one finds from the M+H−CN ion of cyanocobalamin. For cupribalamin no loss of 63 mass units (Cu) is observed. This suggests that the loss of 59 d in cyanocobalamin is not due to extrusion of the cobalt atom but rather to elimination of acetamide. This behaviour is in accord with the finding that it is also very difficult to eliminate the cobalt atom from the corrin system by chemical means [9].

The loss of acetamide rather than cobalt was then unambiguously proven by high-resolution measurement of the ion of mass 1270 for cyanocobalamin. The exact mass was determined to be 1270.5351 which corresponds to the elemental composition C_{60}H_{89}CoN_{12}O_{13}P (theoretical mass: 1270.5350). The ion of mass 1329 (C_{62}H_{89}CoN_{13}O_{14}P = 1329.5721) was used as the reference. Loss of cobalt from the M+H−CN ion would have resulted in an ion of the composition C_{60}H_{89}N_{13}O_{14}P (1270.6389) which was not observed.

### Experimental

The FAB mass spectra were obtained on a Kratos MS 50 S instrument equipped with a Kratos FAB source. The samples were bombarded with 9 kV xenon atoms. Cyanocobalamin was of commercial origin (Fluka). Hydrogenobalamin and cupribalamin were prepared as described by us before [5].

### Table I. Main fragments in the positive ion FAB mass spectra of cyanocobalamin [2], cupribalamin and hydrogenobalamin.

<table>
<thead>
<tr>
<th>Fragment</th>
<th>m/z</th>
<th>Cyanocobalamin</th>
<th>Cupribalamin</th>
<th>Hydrogenobalamin</th>
</tr>
</thead>
<tbody>
<tr>
<td>M+H</td>
<td>1355</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td>1329</td>
<td>(M+H−CN)</td>
<td></td>
<td>1333 (M+H−CN)</td>
</tr>
<tr>
<td>Z−CONH</td>
<td>1286</td>
<td>1290</td>
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<td>1290</td>
</tr>
<tr>
<td>Z−CH2CONH$_2$ (l)</td>
<td>1270</td>
<td>1274</td>
<td></td>
<td>1274</td>
</tr>
<tr>
<td>Z−DMB$^a$</td>
<td>1183</td>
<td>1187</td>
<td></td>
<td>1187</td>
</tr>
<tr>
<td>Q·CH$_2$CH$_2$CONHCH$_3$·</td>
<td>1126</td>
<td>1130</td>
<td></td>
<td>1130</td>
</tr>
<tr>
<td>CH(CH$_3$)OPO$_2$(CH$_3$)$_2$OH$^b$</td>
<td>1069</td>
<td>1073</td>
<td></td>
<td>1073</td>
</tr>
<tr>
<td>Q·CH$_2$CH$_2$CONHCH$_3$·</td>
<td>1051</td>
<td>1055</td>
<td></td>
<td>1055</td>
</tr>
<tr>
<td>CH(CH$_3$)OPO$_2$HOH+H (=Y)</td>
<td>989</td>
<td>993</td>
<td></td>
<td>993</td>
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<tr>
<td>Y−H$_2$O</td>
<td>971</td>
<td>975</td>
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<td>975</td>
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<tr>
<td>Q·CH$_2$CH$_2$CONHCH$_3$·</td>
<td>914</td>
<td>918</td>
<td></td>
<td>918</td>
</tr>
<tr>
<td>CH(CH$_3$)OH (≡X)</td>
<td>859</td>
<td>863</td>
<td></td>
<td>863</td>
</tr>
</tbody>
</table>

$^a$ DMB = 5,6-dimethylbenzimidazole; $^b$ Q = Z minus complete f side-chain.

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