

## ISOLATION OF VITAMIN D<sub>3</sub> FROM NATURAL MUMIYO

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The chemical composition of mumiyo is interesting because it has a wide spectrum of physiological activity.

Many curative properties are ascribed to mumiyo, among these are accelerated healing of bones after breaks and maintenance of phosphorus—calcium exchange [1, 2]. Studies of the vitamin-D activity of mumiyo have also appeared [3].

In order to prepare vitamin D (calciferol), we developed a chemical process to isolate from mumiyo Asil pure vitamin D<sub>3</sub>.

It was found that vitamin D may be bound to lipoproteins and proteins, forming a stable complex. Treatment with base or a dehydrating solvent system is necessary to separate it from the complex [4].

Therefore, we came to the conclusion that vitamin D in mumiyo is strongly complexed to phospholipids, proteins, amino acids, and other nitrogenous bases. Mumiyo was disintegrated by dissolution in NaHCO<sub>3</sub> solution (1 M) and was treated with C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>OCOCl with vigorous stirring (Schotten—Baumann conditions) [5] in order to bind the components of the destroyed complex with released primary and secondary amines and other nucleophilic groups. Stirring was continued for 8-10 h. Extraction with ether removed the excess of C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>OCOCl. The solution was acidified with HCl (6 N) until the pH was 3-3.5. The resulting oily product was extracted with CHCl<sub>3</sub>. The CHCl<sub>3</sub> was removed. The residue was vacuum distilled to afford white crystals as thin needles with mp 83-85°C (Boetius stage, Germany).

Spraying the chromatograms with SbCl<sub>5</sub> solution gives a brownish-blue color typical of the D vitamins. The standard for comparison was vitamin D<sub>2</sub>.

Vitamin D<sub>3</sub> was analyzed by TLC on Silufol UV-254 plates, *R<sub>f</sub>* 0.84 (*n*-butanol:acetic acid:water, 4:1:1), *R<sub>f</sub>* 0.66 (pyridine:isoamyl alcohol, 1:1), *R<sub>f</sub>* 0.94 (CH<sub>3</sub>OH:H<sub>2</sub>O, 98:2). Bands were developed using iodine vapor. The mass spectrum has strong peaks for ions with *m/z* 384 [C<sub>27</sub>H<sub>44</sub>O]<sup>+</sup>, 229 [C<sub>16</sub>H<sub>21</sub>O]<sup>+</sup>, 124 [C<sub>8</sub>H<sub>12</sub>O]<sup>+</sup>, 107 [C<sub>8</sub>H<sub>11</sub>]<sup>+</sup>, 105 [C<sub>8</sub>H<sub>9</sub>]<sup>+</sup>, 95 [C<sub>7</sub>H<sub>11</sub>]<sup>+</sup>, 93 [C<sub>7</sub>H<sub>9</sub>]<sup>+</sup>, 81 [C<sub>6</sub>H<sub>9</sub>]<sup>+</sup>, and 79 [C<sub>6</sub>H<sub>7</sub>]<sup>+</sup>.

The physicochemical properties of the compound isolated by us correspond with vitamin D<sub>3</sub> (cholecalciferol). UV spectrum ( $\lambda_{\max}$ , 268 nm),  $[\alpha]_D^{20} +33^\circ$  (*c* 1.3, CHCl<sub>3</sub>) (Polomate-S, Germany).

## REFERENCES

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