

# Thermal analysis of mumiyo, the legendary folk remedy from the Himalaya region

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## Abstract

Mumiyo is a natural product found mainly in the high mountain ranges of the Himalayas and in some other regions, too. It has been well known as an expensive remedy for a number of diseases for thousands of years. It disappeared completely from the Western medicine and pharmacology, but was frequently used in the former Soviet Union. The present paper concentrates on physical, chemical and microbiological properties of mumiyo, investigated by means of thermal analysis, flow microcalorimetry, capillary electrophoresis, scanning electron microscopy, determination of its natural radioactivity and conventional bioassays with bacteria and fungi. The paper shows that mumiyo is a complex mixture of effective pharmacological substances that acts as a natural bacteriostatic or even bactericidal agent.

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## 1. Introduction

### 1.1. Historical facts about mumiyo

Although known in Europe for more than two millennia and described in old pharmaceutical books, mumiyo is nowadays rather unfamiliar in the West and it is difficult to find scientific and sound information about it. Therefore, the following text contains information taken from several internet sources given before the Acknowledgements. The present paper deals with the results of laboratory investigations on mumiyo, a “secret weapon” against several ailments.

Mumiyo has been known for more than two thousand years, but due to its mysterious origin it might be doubtful that the term denoted the same substance, rather different substances used under the same name. In any case, it was always a very precious remedy reserved for the ruling class, more expensive than gold and in recent times forbidden to be exported from the Soviet Union because it was a

“treasure of the country”. People, mainly in Central Asian regions had different, often very picturesque names for mumiyo like “mountain balsam”, “mountain tears”, “blood of the mountains” or “sweat of the mountains”, to give just a few. Etymologically, the name mumiyo did not originate from the Egyptian mummies but from an old French word “mum” for wax or a soft balsamic resin that was used in Persia and Babylonia. An old dictionary from the 18th century describes mumiyo as a “very precious aromatic and very effective mountain balsam for wound-treatment in Persia”.

In most publications on mumiyo it is mentioned that Aristotle already knew this substance, investigated it and wrote about it. However, an intensive search in the *Corpus Aristoteles* [1] showed that there is no hint to any such substance like mumiyo. It is for sure that there is no Greek word with the root *mum* or alike. The name originated from the Arabic and entered the European languages via the late Latin and Italic. It can be detected since Paracelsus in German pharmacology and moved also into the byzantine Greek as a foreign and loan-word (personal communication by Prof. Diether Roderich Reinsch, Free University of Berlin).

Mumiyo was and still is, mainly found in the high mountain ranges of Central Asia like Pamir, Altai, Hindukush

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and Tsao-Shing, but also mentioned in Japan and Algeria. Special places to look for it are caves and clefts in rocks in remote areas between 2000 and 3500 m with specific weather conditions concerning summer and winter temperatures, duration of sunshine and amount of precipitation.

### 1.2. Physical and chemical properties of mumiyo

Mumiyo is described as a brown to black, sticky and tenacious material with a shiny and polished surface, easily soluble in water, alcohol and acetone. A general description states: 14–20% (humidity); 18–20% (minerals); 13–17% (proteins); 4–4.5% (lipids); 3.3–6.5% (steroids); 18–20% (nitrogen-free compounds); 1.5–2% (carbohydrates); and 0.05–0.08% (alkaloids, amino acids and other compounds with nitrogen) [2]. Other sources are (in  $\text{g kg}^{-1}$ ): potassium 60; calcium 27; magnesium 14; sulphur 6; but sodium only 40 mg. Besides them aluminium, arsenic, barium, iron, fluorine, iodine, copper and manganese. Moreover, diverse amino acids and 65 further organic compounds are listed, among them free fatty acids, waxes, carbohydrates, alkaloids, resins, steroids, polyphenols, essential oils and vitamins. An insignificant natural radioactivity is ascribed to the strontium that is found with  $145 \text{ mg kg}^{-1}$  in mumiyo. It is often stated in the literature that mumiyo contains, besides the above mentioned minerals products and compounds from the root saps of many plants growing near the places where it is found. This corresponds well with the high potassium content determined in mumiyo and the fact that plants contain between 0.5 and 6% potassium [3].

### 1.3. Medical applications of mumiyo

Many medical applications of mumiyo, external as well as internal are cited in the above mentioned web sites, mainly based on observations and studies in the former Soviet Union. Not all of them sound very realistic and serious. Among the most often cited treatments are those for strengthening the immune system and protection against allergies, but also for gastric and intestinal ulcers and healing of fractures. Bilokon and colleagues [4] reported about a stabilising effect on lipid peroxidase during radiotherapy of rats (see also [5]). Spassov [6] compared the effect of mumiyo with that of the well-known nootropic (brain stimulating) drug Piracetam on learning and memory processes of rats in a water maze. Both treatments had highly significant results. But it should be realised that both substances were administered in high doses of  $100 \text{ mg kg}^{-1}$  while the recommended amount of mumiyo for an adult person varies between 50 and 800 mg per day. Respiratory diseases and inflammations are also mentioned, traumata, burns and skin diseases, prophylaxis against osteoporosis, but also as a food additive and as a support against exhaustion, anti-ageing, weariness and stress. Mumiyo is often prescribed in combination with honey as an antiseptic agent. We present microbiological experiments pointing in this direction.

As mumiyo is an extremely complex multi-component natural product, we thought it worthwhile to compare it with another natural product, the bee-glue propolis, which has long been known for similar physiological properties [7]. For several years, propolis from different regions around the world has been investigated in our group for its antimicrobial actions [8]. Thus, we included it in our present thermal investigations to find perhaps similarities between the two and get more information on mumiyo.

## 2. Experimental

### 2.1. Mumiyo source

The applied mumiyo samples were bought on the central bazaar of Almati, the capital of Kazakhstan. Mumiyo is used as a panacea against several ailments in the traditional folk medicine in Kazakhstan as well as many other countries in Central Asia and was a recognised drug sold in the whole of Soviet Union. Nowadays it is allowed to be exported and even to be bought via the internet in the Western world.

### 2.2. Thermal analysis

The thermal behaviour of mumiyo was studied by simultaneous thermal analysis (STA) coupled to a mass-spectroscopic evolved gas analysis (EGA) device. A thermoanalyzer (STA 409 C Skimmer<sup>®</sup> system, Netzsch, Selb, Germany), equipped with a mass spectrometer BALZERS QMG 421 (Balzers, Liechtenstein), was used to record the conventional thermoanalytical curves (T, DTA, TG and DTG) together with the ionic current (IC) curves in the multiple ion detection (MID) mode [9,10]. The MID mode measurements were preceded by scan mode runs covering the mass number range  $m/z$  from 46 to 300 which allowed for the detection of occurring fragments (Table 1). A DTA-TG sample carrier system with platinum crucibles (baker, 0.8 ml) and Pt/PtRh10 thermocouples was used. Mortared samples of 11–18 mg each were measured versus an empty reference crucible in the temperature range 25–700 °C. A constant purge gas flow of  $70 \text{ ml min}^{-1}$  nitrogen (5.0, Messer Griesheim) or of synthetic air (20.5% O<sub>2</sub> 4.8; 79.5% N<sub>2</sub> 5.0) and a constant heating rate of  $10 \text{ K min}^{-1}$  were applied. Raw data were evaluated utilising the manufacturer's software PROTEUS<sup>®</sup> (v. 4.1+) and QUADSTAR<sup>®</sup> 422 (v. 6.02) without further data treatment, e.g. smoothing.

### 2.3. Capillary electrophoresis

To determine the amount of different cations in the mumiyo samples, capillary electrophoresis was performed with a Quanta 4000 Capillary Electrophoresis System (Waters, Eschborn, Germany). Mumiyo samples were prepared in a mortar rendering light to dark brown powders depending upon the origin. Fifty milligram powder was dissolved with shaking during 2 h in  $500 \mu\text{l}$  double-distilled water with ad-

Table 1  
Interpretation of mass numbers  $m/z$  occurring during the thermal degradation of mumiyo

In air	In N <sub>2</sub>	Attribution	Possible sources
17	17	OH <sup>+</sup>	Water
18	18	H <sub>2</sub> O <sup>+</sup>	Water
	38	C <sub>3</sub> H <sub>2</sub> <sup>+</sup>	Aromates
	39	C <sub>3</sub> H <sub>3</sub> <sup>+</sup>	
	40	C <sub>3</sub> H <sub>4</sub> <sup>+</sup> CH <sub>2</sub> CN <sup>+</sup>	
	41	C <sub>3</sub> H <sub>5</sub> <sup>+</sup> CH <sub>3</sub> CN <sup>+</sup>	
	42		Unspecific
	43	CH <sub>3</sub> CO <sup>+</sup>	Acetyl compounds
44	44	CH <sub>3</sub> -CH=NH <sub>2</sub> <sup>+</sup>	Amines
		CH <sub>2</sub> =NHCH <sub>3</sub> <sup>+</sup>	Amines
		CO <sub>2</sub> <sup>+</sup> , CONH <sub>2</sub> <sup>+</sup>	Acids, amides
	45	C <sub>2</sub> H <sub>5</sub> O <sup>+</sup>	Alcoholes
		COOH <sup>+</sup>	Carbonic acids
		CHS <sup>+</sup>	Thioles
	50		
51	C <sub>4</sub> H <sub>3</sub> <sup>+</sup>	Aromates	
55			
67			
68		C <sub>5</sub> H <sub>8</sub> <sup>+</sup>	Monosubstituted benzene derivatives
		C <sub>4</sub> H <sub>4</sub> O <sup>+</sup>	
		C <sub>3</sub> H <sub>6</sub> CN <sup>+</sup>	
	77	C <sub>6</sub> H <sub>5</sub> <sup>+</sup>	
78			
79	79	C <sub>6</sub> H <sub>7</sub> <sup>+</sup>	Aromates with H-containing substituents
		C <sub>5</sub> H <sub>5</sub> N <sup>+</sup>	Pyridines, pyrroles
	91	C <sub>7</sub> H <sub>6</sub> <sup>+</sup>	Alkyl substituted aromates
	92	C <sub>6</sub> H <sub>6</sub> N <sup>+</sup>	Alkyl pyridines
94	94	C <sub>6</sub> H <sub>6</sub> O <sup>+</sup>	Phenol ethers, esters
96			
105	105	C <sub>7</sub> H <sub>5</sub> O <sup>+</sup>	Benzoyl compounds
		C <sub>8</sub> H <sub>9</sub> <sup>+</sup>	Alkylated aromates

ditional 30 min ultrasound treatment. After this, the stock solution was diluted 1:20 and again 1:50, coming to a total dilution of 1:1000. This final solution was filtered for 10 min at 1400 rad s<sup>-1</sup> in a Microcon YM-30/Regenerated Cellulose 30 000 MWG (Amicon, Cat.No. 42410, Millipore, Eschborn, Germany).

#### 2.4. Radioactivity

Several publications on mumiyo mention its low-level natural radioactivity attributed to its content of strontium. Therefore, some measurements were performed on raw and commercial specimens (tablets) refined (in a secret manner by the collectors) before selling. Special attention was paid to the high concentration of potassium also cited in the literature [2]. A multi-channel gamma detector (FHT770m/FHT7000; FAG Kugelfischer Georg Schäfer, Erlangen, Germany) was used in these experiments that were performed in the Institute of Chemistry, Free University of Berlin.

#### 2.5. Scanning electron microscopy

A Scanning electron microscope (Philips SEM 515, Eindhoven, The Netherlands) was used to look for structures and inclusions in mumiyo samples. The high tension was set to 8 kV, the magnification varied in the pictures and can be determined in the pictures by the length of a single white or black bar and the indicated value. Samples were air-dried and gold-sputtered by means of a sputter coating device SCD 040 (Balzers Union, Vaduz, Liechtenstein).

#### 2.6. Microcalorimetric investigations

A combination of a flow calorimeter (type 10700-1, LKB, Bromma, Sweden; flow-through spiral of 0.587 ml; sensitivity 61.6 μV mW<sup>-1</sup>), a two-channel recorder (BD5, Kipp & Zonen, Delft, The Netherlands) for the power–time ( $p-t$ ) curve registration and an external well stirred fermentor with 20 ml active volume were used. The experiments were run at 30 °C and a flow rate of 56 ml h<sup>-1</sup> after sterilising the set up with a solution of 10% H<sub>2</sub>O<sub>2</sub> and 2% H<sub>2</sub>SO<sub>4</sub> in 60% ethanol for 15 min and neutralising with 0.1 M KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub> of pH 7.0 for 30 min.

Four species of gram-positive (*Bacillus brevis*, *Bacillus megaterium*, *Bacillus subtilis* and *Micrococcus luteus*) and two species of gram-negative (*Escherichia coli* and *Pseudomonas syringae*) bacteria were used in the experiments. Two hundred micro litre of an overnight culture were inoculated into 20 ml of a complex growth medium (Standard I nutrient broth, Merck, Darmstadt, Germany). When the bacterial culture was at the mid of the logarithmic growth phase mumiyo was added in changing volumes of the stock solution to come to final concentrations of 1, 2 or 4% (w/v). Control experiments used corresponding volumes of distilled water.

### 3. Results and discussion

#### 3.1. General observations

It is often stated that mumiyo is easily soluble in water. But our own results show that such “solutions” are suspensions with a high amount of floating particles that can be precipitated by centrifugation. Only about 60% of the raw material is soluble. These brownish solutions show a broad absorption peak at 570 nm with an extended shoulder to shorter wavelengths and a steeper decline at the other side and possess pH values between 6.5 and 7.5.

Pills, as well as raw material, have a black, shiny surface and a pleasant and aromatic smell. Our samples did not soften at ambient temperatures but remained hard and brittle so that it was difficult to cut off small specimens. Fig. 1 shows raw mumiyo as obtained from the bazaar, Figs. 2 and 3 shows two SEM pictures at low magnification of few-milligram pieces. They demonstrate that mumiyo is a



Fig. 1. Macro photograph of a piece of mumiyo (5 cm, 10 g) from Kazakhstan.

homogeneous material without (the expected) inclusion of inorganic or organic origin (crystals, grains of sand, pollen, seeds, etc.).

### 3.2. Thermal analysis

The thermal behaviour of mumiyo strongly depends on the gas atmosphere. Typical STA traces in air are shown in Fig. 4. The DTA curves may differ between various heating runs indicating hereby that samples of mumiyo are not uniform but show a batch dependence. These differences in intensity and signal form can be observed especially at higher temperatures. However, this seems to be of minor importance

because the general character of the degradation process, spread over five partially overlapping TG steps, remains uninfluenced. In an oxidising atmosphere, only exothermal processes occur except during the dehydration range up to 150 °C (about 7% H<sub>2</sub>O). This indicates that mumiyo predominantly consists of organic matter. The total mass loss in air amounts to 67.6%.

The IC curves for  $m/z$  17 (OH<sup>+</sup>), 18 (H<sub>2</sub>O<sup>+</sup>) and 44 (CO<sub>2</sub><sup>+</sup>) in air (Fig. 5) show that mumiyo is only dehydrated in the low temperature range. The decomposition processes start at about 160 °C accompanied by an increase in CO<sub>2</sub> formation. This temperature is not too low to be interpreted in terms of the initial decarboxylation of car-

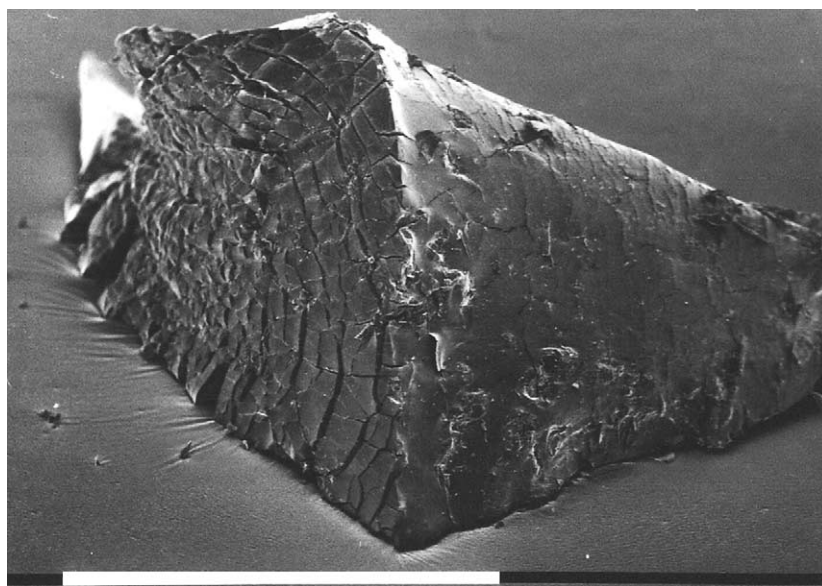


Fig. 2. SEM photograph of a tiny piece of raw mumiyo. Cracks are seen in the sample and the smooth surface. The length of the white bar corresponds to 1 mm.



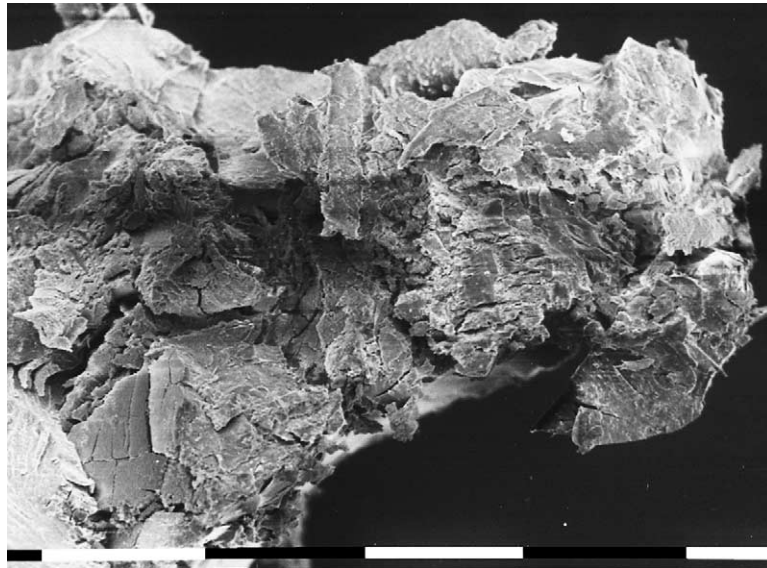


Fig. 3. SEM photograph of another piece of raw mumiyo material. White and black bars correspond to 0.1 mm. No inclusions are observable.

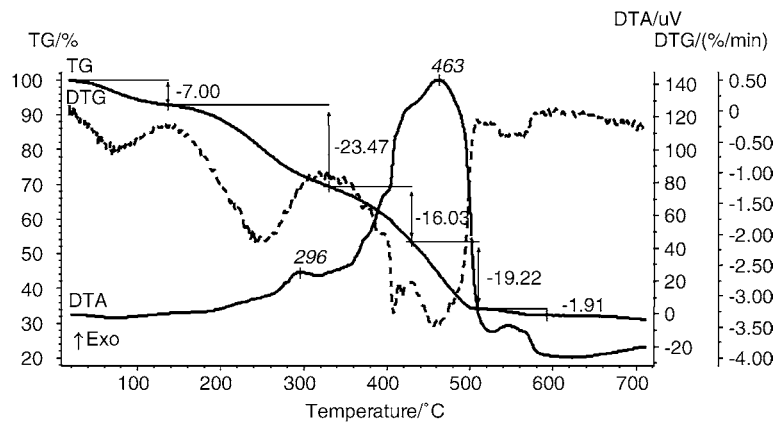


Fig. 4. Simultaneous thermal analysis (STA) curves of mumiyo in air ( $m = 18.32$  mg).

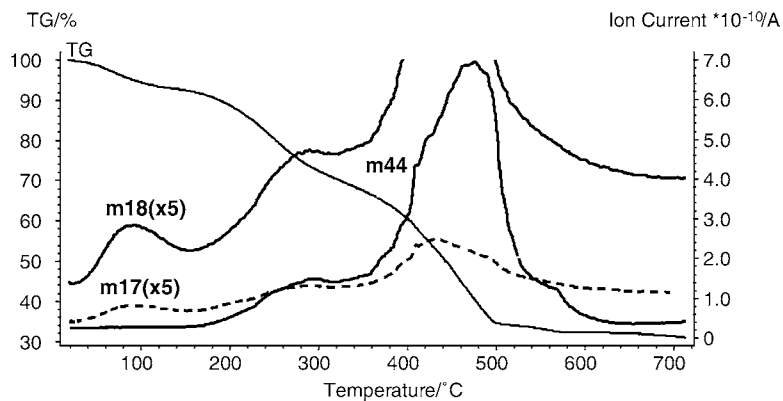


Fig. 5. Ionic current (IC) curves for the mass numbers  $m/z$  17 ( $\text{OH}^+$ ), 18 ( $\text{H}_2\text{O}^+$ ) and 44 ( $\text{CO}_2^+$ ) (to Fig. 4).

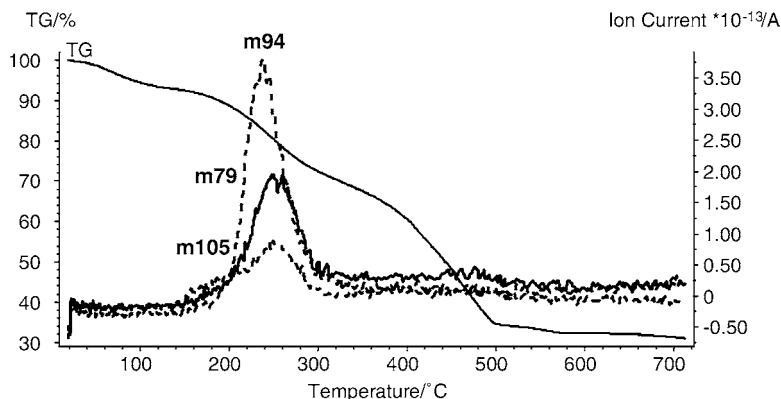
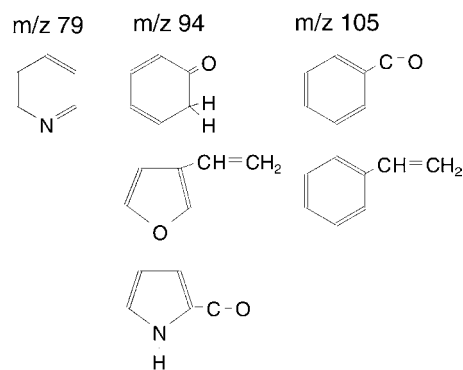


Fig. 6. IC curves for the mass numbers  $m/z$  79, 94 and 105 (to Fig. 4).

bonic acids. Real combustion, which also yields  $\text{CO}_2$  along with  $\text{H}_2\text{O}$ , proceeds at higher temperatures. The IC curve for  $m/z$  44 exhibits two maxima: a weaker one at about  $300^\circ\text{C}$  and a strong one at about  $470^\circ\text{C}$ . Both maxima, however, were preceded by maxima for  $m/z$  79, 94 and 105 at  $240^\circ\text{C}$  (Fig. 6). They presumably represent simple evaporation and/or decomposition of components whereas at higher temperatures, combustion processes predominate. Greater mass numbers than  $m/z$  105 have not been detected in air.

A completely different behaviour is observed in an inert atmosphere (Fig. 7). A TG curve without well-expressed TG steps corresponds to a DTA curve exhibiting only weak endothermic signals which are followed, however, by a strong endothermic effect with  $T_{\text{on}}^{\text{ex}}$   $378^\circ\text{C}$  and  $T_p$   $409^\circ\text{C}$ . Close to the peak maximum, strong foaming of the sample together with coke formation causes an overflowing balance signal. But during this first scan the substance flow of the decomposition products was sufficient for a qualitative detection of a certain number of relevant fragments which are summarised in Table 1. As expected for organic matter, the number of detectable fragments is considerably higher in nitrogen than in air. In nitrogen, evaporation and decomposition predominate whereas combustion, yielding  $\text{CO}_2$  and  $\text{H}_2\text{O}$ , is the main process in an oxidizing atmosphere. As the fragments with  $m/z$  79, 94 and 105 were observed for both



Scheme 1. Scheme for the mass numbers  $m/z$  79, 94 and 105.

atmospheres, they seem to represent essential components of considerable amount. A possible attribution to molecular fragments is shown in the formula Scheme 1. On the other hand, these mass numbers are not specific (see below).

Because of the complex nature of mumiyo, the attribution of the observed mass numbers in Table 1 to substance groups gives just a raw orientation and is not surprising taking into account the reported properties. To get some further information, we compared the mumiyo results with the thermal behaviour of bee glue propolis, which has well-known

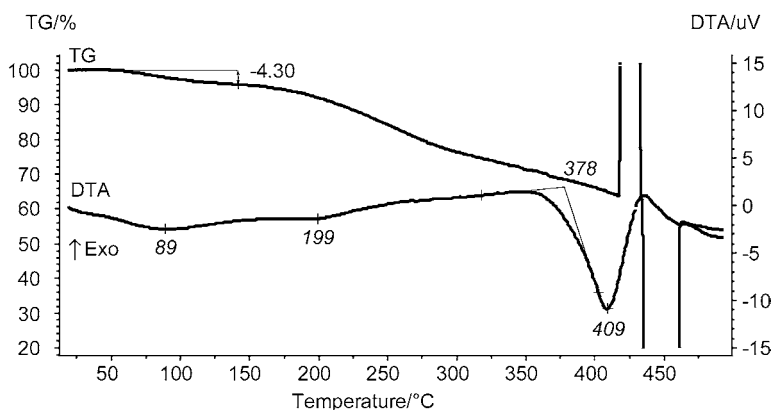


Fig. 7. STA curves of mumiyo in nitrogen ( $m = 11.00$  mg).

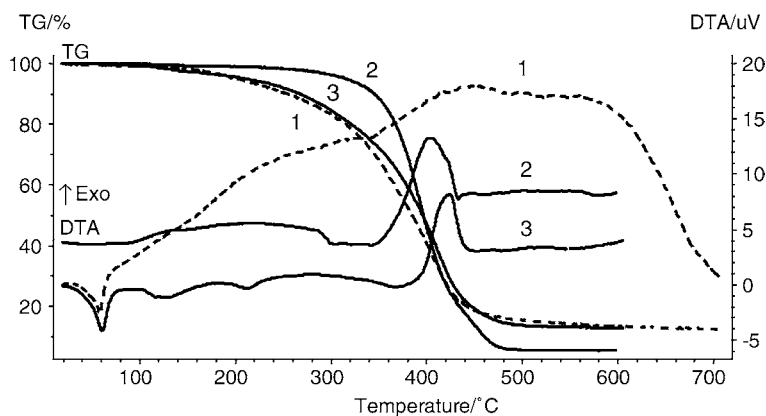


Fig. 8. STA curves of propolis in nitrogen originating from Germany (1), Brazil (2) and South Africa (3).

broad-spectrum physiological properties [7]. Depending on the recipe for propolis solutions (e.g. in water or ethanol) and on its provenance, strong bacteriostatic and bactericidal actions may be observed. It is known that the most important group of organic compounds in this natural drug are flavonoid pigments, frequently found in the plant kingdom. Amino, aliphatic and aromatic acids together with their corresponding esters, alcohols, aldehydes, ketones and terpenoids are further essential components among the known 200 constituents [11,12]. Therefore, propolis was included in the present thermal analytical investigations. Fig. 8 shows the STA curves of propolis originating from three different regions. The TG traces are quite similar whereas the DTA curves differ significantly among another. Only two samples show a distinct melting peak in the low temperature range.

It can be stated that the DTA curves of mumiyo (Fig. 7) and propolis (Fig. 8) in nitrogen deviate considerably from each other. Two basic differences can be deduced. Firstly, the main decomposition step of mumiyo in nitrogen starts with a strongly endothermal effect whereas propolis shows only exothermal phenomena which can be expressed quite differently (batch depending). Secondly, mumiyo has a dehydration range below 150 °C whereas propolis does not. On the other hand, the analysis of the products evolved during the thermal treatment shows certain similarities. Unfortunately, these similarities mainly concern the detection of substances which are practically omnipresent in this kind of multi-component mixture: aromatic carbonic acids, alkylated aromates, alcohols, etc. Phenolic compounds includ-

ing cinnamic acid derivatives and flavonoids constitute the largest (>50 wt.%) fraction of propolis [13–15]. As an example, Table 2 [16] regroups MS data for one of these substance groups, reported to be contained in propolis. It demonstrates that it is impossible to distinguish between the compounds. The mass numbers observed in our measurements are in good agreement with the reported substance classes but do not allow for a more detailed interpretation. In this case of very complex mixtures, EGA data are less appropriate to characterise the samples. Because DTA traces can be regarded as fingerprints, to some extent, conventional STA curves seem to be more promising for comparative studies in this field.

### 3.3. Capillary electrophoresis

Typical cation determinations at 214 nm rendered three prominent peaks and one small peak; in a few runs a second small peak preceded the others. This signal attributed to ammonium may be suspected in all samples, but usually it is too small to be clearly detected. The other peaks are, in the order of their appearance, due to potassium, calcium, sodium and magnesium. Table 3 compiles the obtained molar concentrations each for a raw mumiyo sample and mumiyo pills commercially available in Kazakhstan.

The results obtained with capillary electrophoresis (Table 3) confirm the high potassium concentration in mumiyo already mentioned above or published in the literature and support the idea that components of the nearby

Table 2

Essential mass numbers of various aromatic compounds contained in propolis (from NIST library data [16])

Substance	<i>m/z</i>							
Cinnamic acid	51	77	103	147	148(M <sup>+</sup> )			
Cinnamic alcohol	51	77	78	91	92	105	115	134(M <sup>+</sup> )
Cinnamic aldehyde	51	77	78	103	131	132(M <sup>+</sup> )		
Isoferulic acid	51	77	105	133	179	194(M <sup>+</sup> )		
3,4-Dimethoxycinnamic acid	77	91	103	105	193	208(M <sup>+</sup> )		
<i>p</i> -Coumaric acid	91	147	164(M <sup>+</sup> )					
Benzoic acid	51	77	105	122(M <sup>+</sup> )				

Table 3

Cation concentrations (mmol) in two mumiyo samples from Kazakhstan, determined by capillary electrophoresis (n.d. is non-detectable)

Sample	Ammonium	Potassium	Calcium	Sodium	Magnesium
Raw mumiyo	n.d.	230.4	30.1	n.d.	36.9
Mumiyo tablets	8.3	260.2	39.0	n.d.	42.9
Mumiyo	n.d.	142.4	69.9	0.13	53.9
Tablets	n.d.	154.8	69.6	n.d.	51.4

plants and roots arrive at the mumiyo because plants contain high amounts of potassium (0.5–6% [3]). The value of 260.2 mmol potassium transforms into 10.4 g in 100 g raw mumiyo, that of the refined sample to 5.8 g. This latter value corresponds well to the 60 g kg<sup>-1</sup> given in the internet literature, supposedly also for a sample treated before selling. All the other cation concentrations are significantly smaller if detectable at all. Calcium renders 1.4 and 2.8 g per 100 g, respectively, while the internet literature presents 27 g kg<sup>-1</sup>. Magnesium comes to 0.97 g and 1.3 g per 100 g, respectively, the internet literature value being 14 g kg<sup>-1</sup>. The sodium concentration is very low, with 3 mg per 100 g near to the detection limit, compared with an internet literature value of 40 mg kg<sup>-1</sup>. No data are found for ammonium that renders 140 mg per 100 g in our experiments, also at the detection limit.

### 3.4. Natural radioactivity

Mumiyo is reported to show a low natural radioactivity which is assumed to be stimulating for cell growth, regeneration and metabolism, perhaps in the sense of hormesis [17]. The high content of potassium (60 g kg<sup>-1</sup> mumiyo [2]) seems to be responsible for the radiation since the stable potassium isotope <sup>39</sup>K is accompanied by the radioactive isotope <sup>40</sup>K (0.0118%). To check this assumption, the radiation of mumiyo was determined in a multi-channel gamma detector (FHT770m/FHT7000; FAG Kugelfischer Georg Schäfer, Erlangen, Germany) and compared with that of normal and dietary table salt and pure potassium chloride (see Table 4), because <sup>40</sup>K is the main internal radiation source in humans and taken up daily with table salt. In dietary table salt sodium is exchanged against potassium with a content of 84 g KCl

Table 4

Radioactivity of raw and purified mumiyo from Kazakhstan compared with that of table salts and pure chemical compounds (n.d. is non-detectable and \* indicates calculated from the assumed amount of K)

Material	Radioactivity counts (min g <sup>-1</sup> )	Radioactivity counts (min g <sup>-1</sup> kg <sup>-1</sup> )
Mumiyo, raw material	37	14*
Mumiyo, purified	6	14*
Table salt	n.d.	n.d.
Dietary table salt	162	368
Potassium chloride p.a.	124	236
Sodium chloride p.a.	n.d.	n.d.

per 100 g. All measurements were performed near the detection limit of the gamma detector so that the astonishing difference between KCl p.a. and dietary table salt is not significant. Table 4 demonstrates that the low observed radioactivity of mumiyo can be connected with the high content of potassium in it. If this radiation might be stimulating in the sense of hormesis remains an open question. All naturally occurring strontium isotopes are stable so that this element does not contribute to the irradiation as supposed in the literature.

### 3.5. Microcalorimetric investigations

Fig. 9 presents power–time curves of growing *Bacillus brevis* cultures under the influence of different mumiyo concentrations. Arrows at the curves mark the addition of mumiyo. The control slope is characterised by a short lag phase and a quick transition into the exponential (logarithmic) phase that leads to a maximum followed by a steep decline to a level that remains constant over a long period of time. The little secondary peak during the drop-off is due to a further energy source metabolically formed during the initial phases.

The three other curves show immediate reductions in the volume specific heat production rates after the addition of mumiyo. These decreases are partly due to dilution effects during addition of mumiyo, but to a larger extent to a poisoning effect. This becomes evident by the strongly diminished incline of the growth curve of the culture (Fig. 9ii) and by the new lag phases in the two other curves. They represent the bacteriostatic effect of the drug that allows metabolism of maintenance but no growth. It takes some time until a resistant sub-population develops to such a concentration that it becomes detectable by the calorimeter. Nevertheless, all three power–time curves with mumiyo come to a pronounced maximum and a consequent drop to the same metabolic level as the control (indicated in Fig. 9ii).

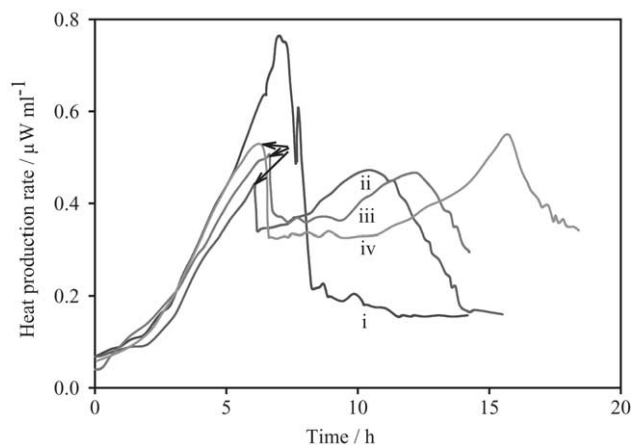


Fig. 9. Heat production rates of four *Bacillus brevis* cultures under the influence of mumiyo. (i) control, (ii) 1% mumiyo, (iii) 2% mumiyo, (iv) 4% mumiyo. Arrows indicate the addition of mumiyo.



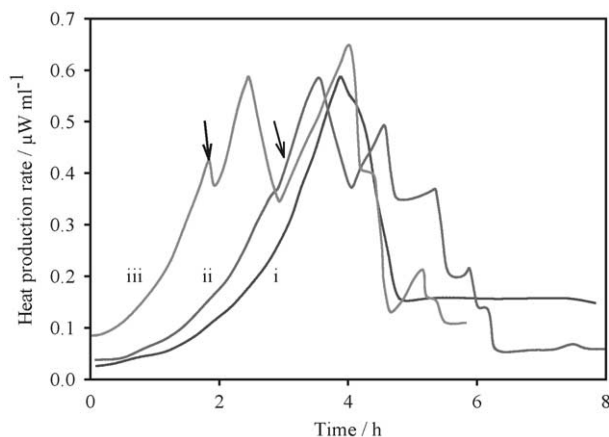


Fig. 10. Heat production rates of three *Bacillus subtilis* cultures under the influence of mumiyo. (i) control, (ii) 2% mumiyo, (iii) 4% mumiyo. Arrows indicate the addition of mumiyo.

A different effect of mumiyo is seen in Fig. 10 with three cultures of *Bacillus subtilis*. The control curve (i) corresponds to that of *B. brevis* shown above with the slight difference that the secondary peak is less marked and visible just as shoulder on the right flank. Addition of mumiyo (arrow) at a final concentration of 2% remains without dilution effect in curve (ii) but several additional peaks appear before the heat production drops to a level near to zero. Finally, curve (iii) with 4% mumiyo brings the dilution effect after addition (arrow) and also a number of secondary peaks that are produced by stepwise decomposition of the primary energy source and further consumption of metabolic products. In any case it becomes clear that there is no immediate killing effect with mumiyo, at best some bacteriostatic activities.

Parallel non-calorimetric experiment, not dealt with here, following the classical Petridish approach will be published in [18]. On an agar plate inoculated with bacteria growth-inhibition halos develop around a central well containing mumiyo in different concentrations. The final halo diameter is directly related to the drug activity. These results show that mumiyo is more effective against gram-positive than gram-negative bacteria, leading to the assumption that mumiyo attacks the peptidoglycan cell wall of the former as most antibiotics that are differently active against both groups of bacteria. Other targets like cell membranes may also be involved.

Petridish assays are integrative methods showing the result only at the end of the experiment. In contrast, calorimetry works “on-line” and shows the momentary metabolic status of a colony. Instead of the one-figure result of the halo diameter the power–time curve exhibits a fingerprint like temporal structure of the microbial heat output and thus metabolism as clearly shown in the two figures presented above. Moreover, influences of low mumiyo concentrations without visible effects in the Petridish assay become evident in the calorimetric curves demonstrating that the different

components of mumiyo become active at different states of the culture (secondary peaks in Fig. 10). An intensive discussion of these biological questions can be found in [18].

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