Half a Century of Spectroscopy on Studentski trg (Belgrade)*

*Faculty of Chemistry and Center of Chemistry – Institute for Chemistry, Technology and Metallurgy, University of Belgrade

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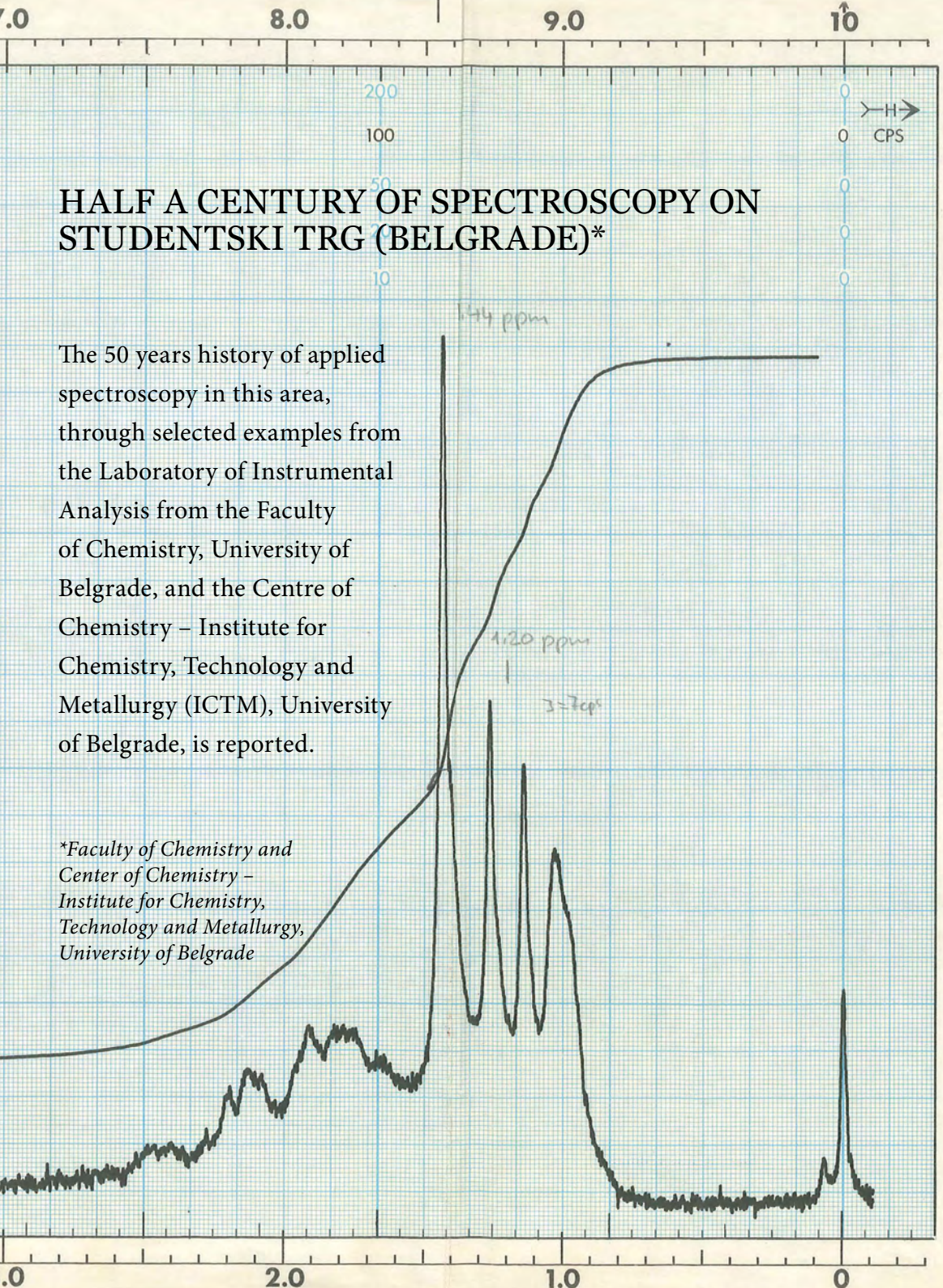
HALF A CENTURY OF SPECTROSCOPY ON STUDENTSKI TRG (BELGRADE)*

The 50 years history of applied spectroscopy in this area, through selected examples from the Laboratory of Instrumental Analysis from the Faculty of Chemistry, University of Belgrade, and the Centre of Chemistry – Institute for Chemistry, Technology and Metallurgy (ICTM), University of Belgrade, is reported.

**Faculty of Chemistry and Center of Chemistry – Institute for Chemistry, Technology and Metallurgy, University of Belgrade*



60 MC NMR SPECTRUM NO. 1269

OPERATOR: M.T. DATE 10. XII. 1970

SAMPLE:

"A"

SOLVENT	$CDCl_3$				
TEMPERATURE	R.T.				°C
FILTER BANDWIDTH	4				cps
R.F. FIELD	0,03				mG
SWEEP TIME	500				sec
SWEEP WIDTH	500				cps
SWEEP OFFSET	000				cps
SPECTRUM AMP.	25				
INTEGRAL AMP.	2,5/80				

REMARKS:

Ardemisia Amruso.

A

Figure 1. The building of the Faculty of Chemistry, University of Belgrade, and the Centre of Chemistry – Institute for Chemistry, Technology and Metallurgy (ICTM), University of Belgrade (Studentski trg 12 – 16).

HISTORICAL

Short time after foundation, 50 years ago, in November 1966, the Laboratory was equipped with the modern analytical instruments such as: nuclear magnetic resonance (^{1}H NMR) spectrometer, infrared (IR) and ultraviolet-visible (UV-Vis) spectrometers, as well as analytical and preparative gas chromatographs (GC). In those days such equipment was to be found in the most prominent European laboratories only.

Prof. Milutin Stefanović 1924-2009

The Laboratory was founded by two institutions: the Institute for Chemistry, the Faculty of Science (today the Faculty of Chemistry), University of Belgrade and the Department for Organic Synthesis (today the Centre of Chemistry) ICTM, University of Belgrade (Figure 1). The first Head of the Laboratory for Instrumental Analysis was Dr. Dragoslav Jeremić and the Head of the Deprtament for Organic Synthesis was Professor Milutin Stefanović.

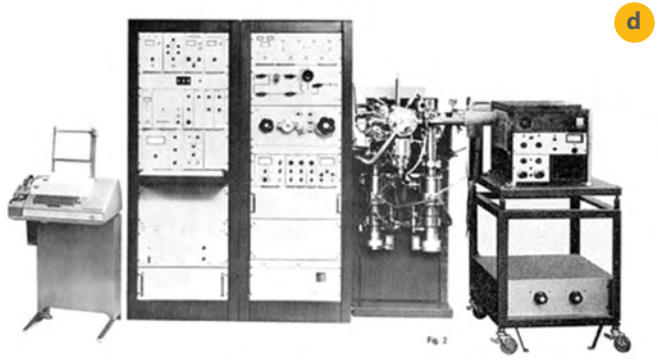
Prof. Dragoslav Jeremić 1929-2011

The Laboratory equipment was completed at the end of 1969 by acquisition of the mass spectrometer which led to the finalization of the modern Center for Instrumental Analysis (CIA, also refered today by this abbreviation), unique in this part of Europe. Putting this equipment into operation, available to all those from the whole former Yugoslavia interested in this field of analysis, had the great impact on the chemistry in the area, raising the general level of scientific work and teaching. The impact was also made on industry by solving different practical problems, e.g. quality control of raw materials and final products in chemical industry, forensic analyses, etc. In addition, thanks to the new equipment, new research areas, such as phytochemistry, were initiated. The Laboratory has survived till nowadays, passing through all the problems concerning the whole society. The worn-out equipment has been replaced by the new one in accordance with available funds. The last big renewal of the instruments has been carried out more than nine years ago in the course of National Investment Plan (NIP) by the

^{1}H 60 MHz NMR (Varian A60A)

IR grating Perkin Elmer model 337

Varian Aerograph GC

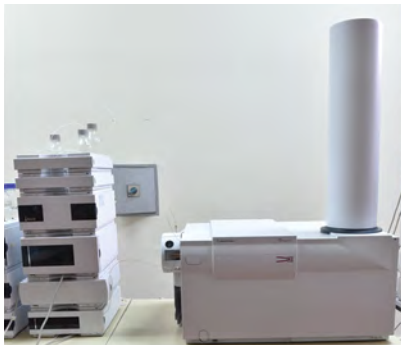


GC/MS Varian MAT CH5

Figure 2. The Equipment of the Laboratory for Instrumental Analysis by the end of 60s.
(a) nuclear magnetic resonance (NMR) spectrometer, (b) IR spectrometer,
(c) gas chromatograph and (d) mass spectrometer.

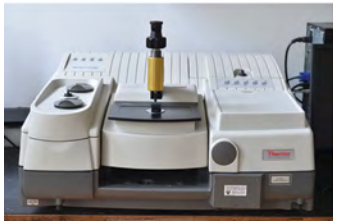
GC-MS

HPLC



LC-ESI-TOF-MS

LC-QqQ-MS



FTIR

UV-Vis

C, H, N, S, O analyzer

500 MHz NMR

200 MHz NMR

Figure 3. Current equipment of the Laboratory for Instrumental Analysis.

Ministry of Science and Technological Development. The following instruments have been supplied: NMR spectrometer (500 MHz for protons), IR spectrometer (FTIR), liquid chromatograph coupled to mass spectrometer (LC/ESI ToF MS) and gas chromatograph coupled to mass spectrometer (GC/MS).

Nowadays the Laboratory for Instrumental Analysis, accredited to ISO 17025 Standard, is a part of the Centre of Chemistry – ICTM and the Faculty of Chemistry, engaging 20 persons. The Laboratory is managed by prof. Vlatka Vajs (scientific adviser, ICTM) and prof. Vele Tešević (Faculty of Chemistry).

ACTIVITIES OF THE LABORATORY

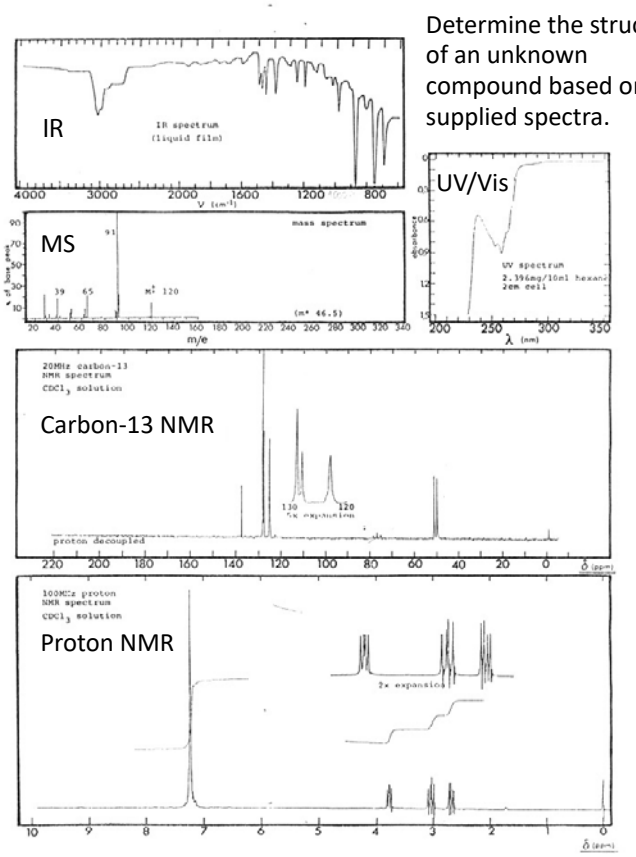
1. Service

Routine spectra, chromatograms (IR, NMR, MS, GC/MS and LC/MS), and microanalyses are being run on a service basis for other groups of the Faculty of Chemistry and Centre of Chemistry, as well as for external users from different institutions (universities, research institutes, industrial laboratories and many others) all over Serbia, mostly free of charge or with compensation of basic expences. Staff members are available for consultations regarding experimental measurements and interpretations of the results. So far hundreds of thousands spectra and chromatograms have been measured for different users.

2. Teaching

The teaching activity involves *Structural Instrumental Methods* for undergraduate students of chemistry and biochemistry, as well as the related advanced courses at master and Ph.D. studies. The Laboratory staff also teach *The Chemistry of Secondary Metabolites and Chemical Weapons* at Ph.D. studies of chemistry. The course *Structural Instrumental Methods* was introduced in 1969 by professor D. Jeremić (initially entitled *Instrumental Organic Analysis*) after the similar course at the Federal High Technical School (ETH) in Zürich. The objective of the course is to teach student to identify an organic compound using MS, IR, UV-Vis, ^{1}H and ^{13}C NMR spectra. Figure 4 is showing the textbooks for the course *Structural Instrumental Methods*, and figure 5 is illustrating an example of examination paper, *i.e.* the spectral problem for the same course.

Figure 4. The textbooks for the course *Structural Instrumental Methods* published by the Faculty of Chemistry, University of Belgrade.



Determine the structure of an unknown compound based on its supplied spectra.

Figure 5. An example of examination paper for *Structural Instrumental Methods* intended for undergadute chemistry students (IR: infra red spectrum, MS: mass spectrum, UV-Vis: ultraviolet-visible spectrum, NMR: nuclear magnetic resonace spectra of carbon-13 and protons).

3. Scientific works

3.1. Phytochemical examinations of the wild-growing plant species of Serbia and Montenegro

Members of the Laboratory for Instrumental Analysis are mainly involved in the phytochemistry research projects financed by the Ministry of Education, Science and Technological Development. These phytochemical examinations originated from collaboration with the group of professor M. Stefanović from the Faculty of Chemistry, who initiated them together with professor D. Jeremić by the end of 60s and was engaged in these research till the end of his career (beginning of this century). These phytochemical investigations, intensified in the Laboratory since 90s, mainly included mountain plant species apart from those from Deliblato Sand (Deliblatska peščara), and were focused on endemites belonging to families and genera known for the species with medicinal properties. Due to multidisciplinary nature of these works, the Laboratory for Instrumental Analysis has established collaboration with quite a few different institutions:

- *The Institute for Medicinal Plant Research "Dr. Josif Pančić", Belgrade*
- *Mayo Clinic, Department of Biochemistry and Molecular Biology (Rochester, Minnesota, dr Slobodan Macura)*
- *Faculty of Biology, University of Belgrade*
- *Botanical Garden Dulovine, Kolašin, Montenegro (Danijel Vincek)*
- *Botanical Garden "Velemun", Plav, Montenegro (Milutin Praščević)*
- *Faculty of Pharmacy, University of Belgrade*
- *Department of Chemistry, Faculty of science, University of Niš*
- *Institute for Biological Research "Siniša Stanković", Belgrade*
- *School of Medicine, Uneversity of Belgrade*
- *Institute for Oncology, University of Belgrade*
- *Institute for Nuclear Sciences, Vinča*
- *Faculty of Science, University of Podgorica, Montenegro*
- *Faculty of Agriculture, University of Belgrade*
- *National Park Galičica, Ohrid, Macedonia*
- *Bulgarian Academy of Science, joint project with Serbian Academy of Science and Arts: "Secondary metabolites from wild-growing and cultivated plants with potential biological activity"*

As far as the scope of collaboration is concerned, the dominant place could be assigned to the *Institute for Medicinal Plant Research "Dr. Josif Pančić", Belgrade* (reffered further as *IMPR "Dr. Josif Pančić"*), *Faculty of Biology* (*Botanical Garden "Jevremovac"*) and *Mayo Clinic* (*Rochester, Minnesota*).

The very important form of collaboration with the IMPR "Dr. Josif Pančić" are pharmacognostic excursions involving study of the mountain flora of Serbia and Montenegro. These excursions have a long tradition in the IMPR "Dr. Josif Pančić" since they have been organized almost every year since the foundation of the Institute. The associates from the Faculty of Chemistry and the Center of Chemistry – ICTM have joined that excursions since 90s (see figure 6).

Figure 6. The picture from pharmacognostic excursion organized by the IMPR "Dr. Josif Pančić", photographed in the year 2002 at Prokletije mountains (Kotlovi) (Photo Tasić).

The most important localities visited and pharmacognostically studied in detail are: Šar planina (Šara mountain) (Kosovo), Stara planina (Old mountain), mountains Tara and Kopaonik in Serbia, Prokletije, Visitor, Zeletin, Komovi, Bjelasica, Sinjajevina, Durmitor, Žabljak, Lovćen, Orjen and Hajla in Montenegro. In the course of these pharmacognostic investigations organized by the IMPR "Dr. Josif Pančić" in last two decades, in Serbia (together with Šara mountain) *ca*. 1100 plant species were registered, and from pharmoco-economic point of view about hundred of them appeared to be interesting. Out of 850 – 900 plant species registered in Montenegro, *ca*. 120 have been phytochemically investigated including those investigated by the group of M. Stefanović (between 1970 and the beginning of 90s).

The list of plant families chemically investigated starting from 70s on the Faculty of Chemistry, the Center of Chemistry – ICTM, and the IMPR "Dr. Josif Pančić" (the number of investigated species is given in parentheses)

1. **Compositae (Asteraceae)**
   Artemisia L. (9), *Ambrosia* L. (1), *Tanacetum* L. (5), *Telekia* Baumg. (19), *Eupatorium* L. (1), *Achillea* L. (10), *Centaurea* L. (15), *Anthemis* L. (6), *Amphoricarpos* Vis. (2), *Senecio* L. (7), *Helichrysum* Mill. (2), *Cicerbita* Wallr. (2), *Ptilostemon* Cass. (2)

2. **Apiaceae (Umbelliferae)**
   Laserpitium L. (4), *Peucedanum* L. (1), *Angelica* L. (1), *Seseli* L. (2), *Chaerophyllum* L. (1), *Athamanta* L. (1), *Mallabaila* Hoffm. (1)

3. **Hypericaceae**
   Hypericum L. (9)

4. **Gentianaceae**
   Swertia L. (2), *Gentianella* Moench. (5), *Gentiana* L. (9)

5. **Dipsacaceae**
Cephalaria Schrad. (3)

6. **Lamiaceae**
Phlomis L. (1), *Satureja* L. (2), *Lycopus* L. (1), *Sideritis* L. (2)

7. **Euphorbiaceae**
Euphorbia L. (2)

8. **Fabaceae**
Trifolium L. (2), *Onobrychis* Mill. (1)

9. **Anacardiaceae**
Cotinus Adans. (1)

10. **Boraginaceae**
Rindera Pall. (1)

11. **Betulaceae**
Alnus Hill. (2)

12. **Pinaceae**[a)]
Pinus L. (3), *Picea* A. Dietr. (1), *Pseudotsuga* Carrière (1)

[a)] Chemotaxonomic investigations under the supervision of prof. Petar Marin (Faculty of Biology, University of Belgrade)

3.1.1. Selected examples

(i) ***Artemisia annua* L. (Fam. Compositae)**

Artemisia annua (sweet wormwood, sweet annie, sweet sagewort, annual mugwort or annual wormwood) is a weed which could be found around the roads, railroads, slopes of embankments, cultivated fields, gardens, around the houses and neglected places and lands. *A. annua* is common in southeast Europe and temperate Asia. It is naturalized in middle and south Europe and North America. It belongs to Euroasian floral element. It is widespread in Serbia. This species was described by J. Pančić in his *Flora of the Principality of Serbia* (1874) as *A. annua* Willd.

Figure 7. *Artemisia annua* L.

A. annua was among the first species chemically investigated by M. Stefanović and D. Jeremić. The isolation of secondary metabolites from this species was the subject of Ph.D. thesis of Abdulaziz Behbud from Afganistan, the graduate student of M. Stefanović. In course of these works from the $CHCl_3$ extract of the air-dried aerial parts of *A. annua*, collected at the locality Staro sajmište (Old Fairgrounds) in Belgrade at October 1970, two new sesquiterpene lactones, arteannuin A (**1**) and arteannuin B (**2**) have been isolated. This investigation was carried in collaboration with D. Jeremić who has measured and interpreted most of the spectra of these compounds. Based on ^{1}H NMR, MS, IR, and optical rotatory dispersion (ORD) both compounds were assigned as the first members of the new cadinane class of the sesquiterpene lactones. Their structures are shown in figure 8.

These results were reported in 1972 at the *8th International Symposium on the Chemistry of Natural Products* in New Delhi. Whereas the epoxide structure of lactone **2** was subsequently confirmed in two laboratories (from Switzerland and USA), the proposed ozonide structure of the second lactone (**1**) was slightly corrected a couple

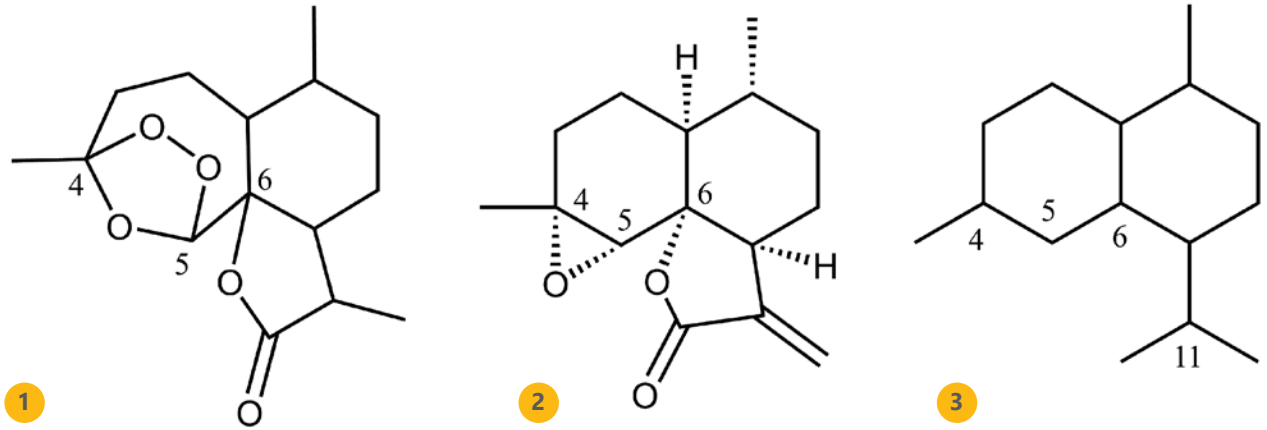


Figure 8. Proposed structures of arteannuin A (**1**) and arteannuin B (**2**); **3**: cadinane skeleton.

of years later (1975) in China. The Chinese proposed a structure **1A** with endoperoxide bridge (figure 9). The structure **1A** was correct, since it was supported by the stronger evidence, such as X-ray diffraction and C-13 NMR, lacking in the case of ozonide structure **1** proposed by Jeremić and Stefanović. It also should be noted that this compound was isolated in China from *A. annua* in course of a highly classified project (***Project 523***) aimed to discover new antimalarial drug, involving more than 500 scientists from ca. 60 different institutions all around China. The use of this plant (named *qinghao*) was featured prominently in Chinese pharmacopoeia in relation to decoctions used to treat fevers including malaria (recorded use of *qing hao* spans over 2000 years). Compound **1A** exhibited extraordinary activity against *Plasmodium falciparum*, the protozoa causing malaria, resistant to standard antimalarics (quinine and chloroquine). Compound **1A** was named ***qinghaosu*** according to the Chinese name of the plant ***qing hao***, as well as *artemisinin* (according to the Latin name of the plant).

The discovery of artemisinin led to the revolution in curing malaria. Nowadays, artemisinin is one of the most efficient antimalarial drugs. Using simple chemical transformations, artemisinin was converted to derivatives (artesunate, artemether, artelinic acid etc.) exhibiting even stronger antimalarial activities

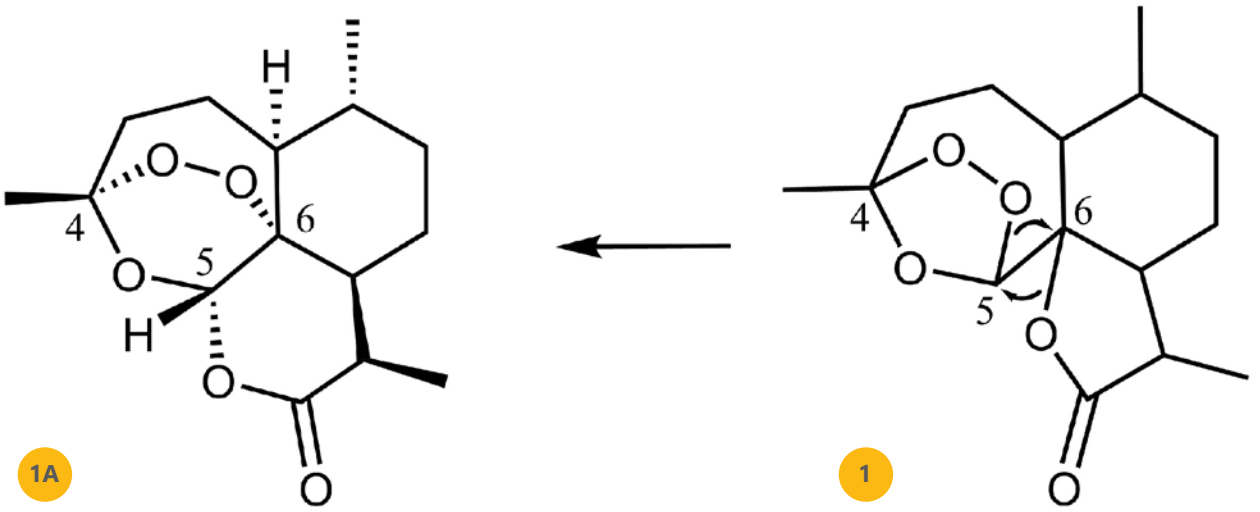


Figure 9. Interchanging positions of oxygen atoms at C-6 and C-5 in structure **1** of arteannuin A (Jeremić and Stefanović) leads to the correct structure of artemisinin (**1A**) proposed in China.

than artemisinin itself. The main source of artemisinin is still cultivated *A. annua*, because the synthesis is complicated and not cost effective.

The importance of this compound that saved hundreds of millions of lives of people infected with malaria was recognized in 2015 by the Nobel Prize for physiology or medicine awarded to Chinese scientist Yoyou Tu, who isolated artemisinin "*for her discoveries concerning a novel therapy against Malaria*", *i.e.* for isolation of ***artemisinin***. She shares Nobel Prize with William C. Campbell and Satoshi Ōmura awarded "*for their discoveries concerning therapy against infections caused by roundworm parasites, nematodes*", *i.e.* for their discovery of macrocyclic lactone of bacterial origin (*Streptomyces*) named ***avermectin***.

(ii) ***Centaurea derventana* Vis. et Panč. (Fam. Asteraceae)**

Centaurea derventana (Derventa knapweed) is an endemic species of Balkan Peninsula. The species is universally distributed in west Serbia and east Bosnia, and its *locus classicus* is on carbonate rocks over brook Derventa close village Rastište (mountain Tara). It was discovered by J. Pančić in 1865, and this was published in: De Visiani, Roberto, Pančić, J.: *Plantae serbicae rariores aut novae. A Prof. Roberto de Visiani et Prof. Josepho Pančić descriptae et icinibus illustratae. Decas II.* – Typis J. Antonelli edit., Venetiis, 1866, 18 pp., Tab. VIII – XV. (Ex Vol. XII, Memor. Imp. Reg. Institut). Pančić also quoted the species in *Flora of the Principality of Serbia* (1874).

Figure 10. *Centaurea derventana* Vis. et Panč. (Photo U. Buzurović).

Secondary metabolites of *C. derventana* were studied in course of systematic investigation of genus *Centaurea* from Serbia and Montenegro as a subject of two Ph.D. theses – at the Faculty of Chemistry as well as the Faculty of Biology, University of Belgrade. In the extract of the aerial parts of the species collected in canyon of brook Derventa in 1996, four structurally close sesquiterpene lactones with germacranolide skeleton were identified: cnicin (**1**), cnicin-4'-*O*-acetate (**2**), salonitenolide-8-*O*-(4'-acetoxy-5'-hydroxy)angelate (**3**) and salonitenolide (**4**) (figure 11), as well as two flavones: apigenin and eupatillin.

The main constituent of the extract (0.2%, calculated per weight of the dried plant material) was cnicin (**1**). This compound is used as a bitter tonic exhibiting so called bitterness value of approximately 1,500 units for bitterniss. Cnicin also shows: cytotoxic, cytostatic and antibiotic activities. The main source of cnicin is *Cnicus benedictus* L. (Asteraceae), the *holy thistle*, but can also be found in many members of genus *Centaurea*.

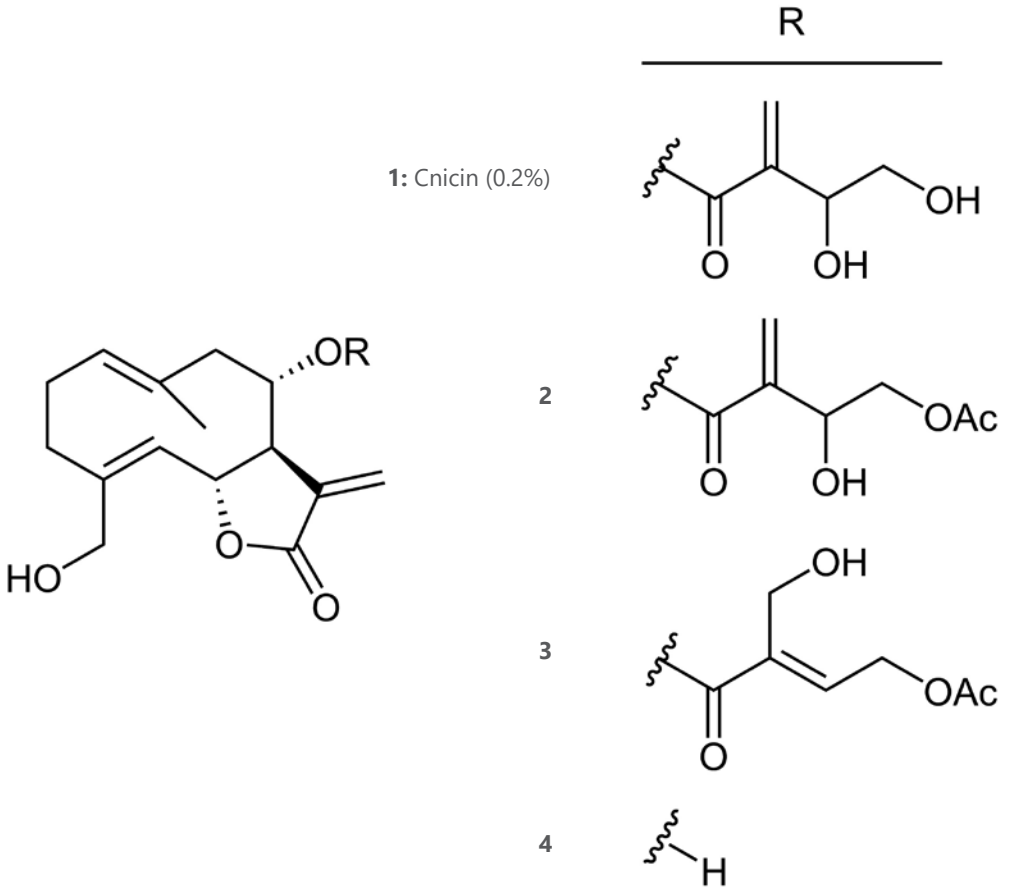


Figure 11. Sesquiterpene lactones isolated from *Centaurea derventana*.

(iii) ***Tanacetum larvatum* (Griesb. ex Pant.) Kanitz. (Fam. Asteraceae)**

T. larvatum is a highland plant species distributed on elevated rocky (carbonate) terrains in Kosovo, Albania and Montenegro. Josif Pančić has found this species in course of his visit to Montengro in 1873, in the rocky area of mountain Durmitor and at Mrčenov do under the mountain Kom. In *Elenchus Plantarum Vascularum quas aestate a. 1873 in Crna Gora* (1875) he quotes this species as *Chrysanthemum larvatum* Griseb. The plant material investigated in our laboratory was collected at mountains in Montenegro: Visitor, Zeletin, Komovi, Sinjajevina and Prokletije (several locations). It also shoud be noted that this species is accidentally omitted from the edition *Flora Europea*. The quantitative ^{1}H NMR of the crude extracts revealed the high content (up to 2%, calcd. per weight of the dried plant material) of the germacranolide sesquiterpene lactone - *parthenolide* (figure 13).

Figure 12. *Tanacetum larvatum* (Griesb. ex Pant.) Kanitz. (Photo Šavikin).

This result is important due to the fact that parthenolide is the main constituent in the current preparations (in form of pills and tinctures) produced from the extracts of the aerial parts of the related cultivate species *Tanacetum parthenium* (named Feverfew). These preparations, based on traditional medicine, are used for the migraine relief, to help prevent blood clots, as an anti-inflammatory providing relief in cases of arthritis, to relieve some types of menstrual problems, and as a digestive aid.

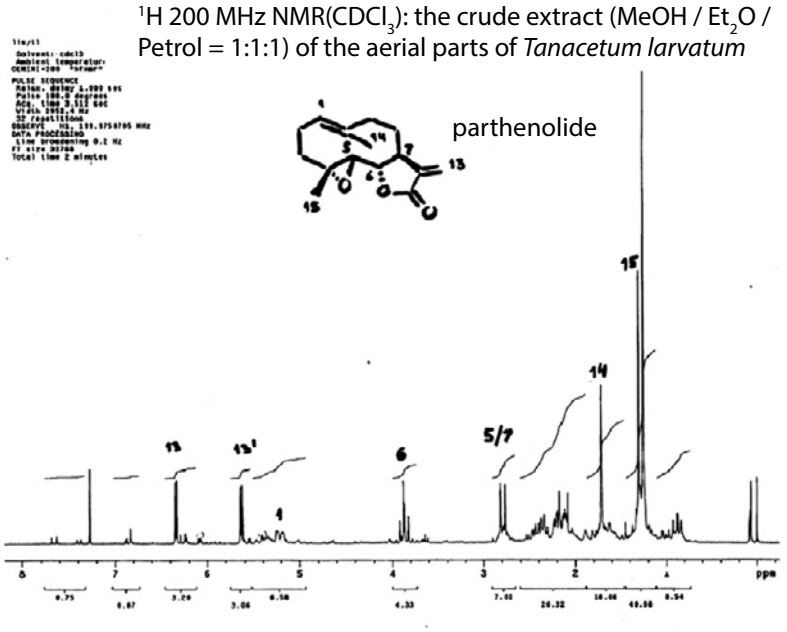


Figure 13. The main signals in the proton NMR spectrum of the crude extract of *T. larvatum* originate from parthenolide.

3.2. Polyphenolic content and antoxidative properties of berry fruit seeds (recent results)

Recently, our investigations have been extended to investigation of new sources (insuficiently used before) of pharmacologically active substances from waste-products of food industry as well as production of alcoholic beverages, such as seeds of different cultivars of grapes, raspberries, blackberries and currants. For example, the application of hyphenated chromatographic and spectroscopic methods (LC/UV/ESI-ToF MS and NMR) revealed a high polyphenolic content in the extracts of grape seeds, the waste material in wine production in our country. The identified polyphenols exhibit antioxidant activity, effective in the prevention of oxidative lymphocyte damage by reactive oxygen species (ROS).

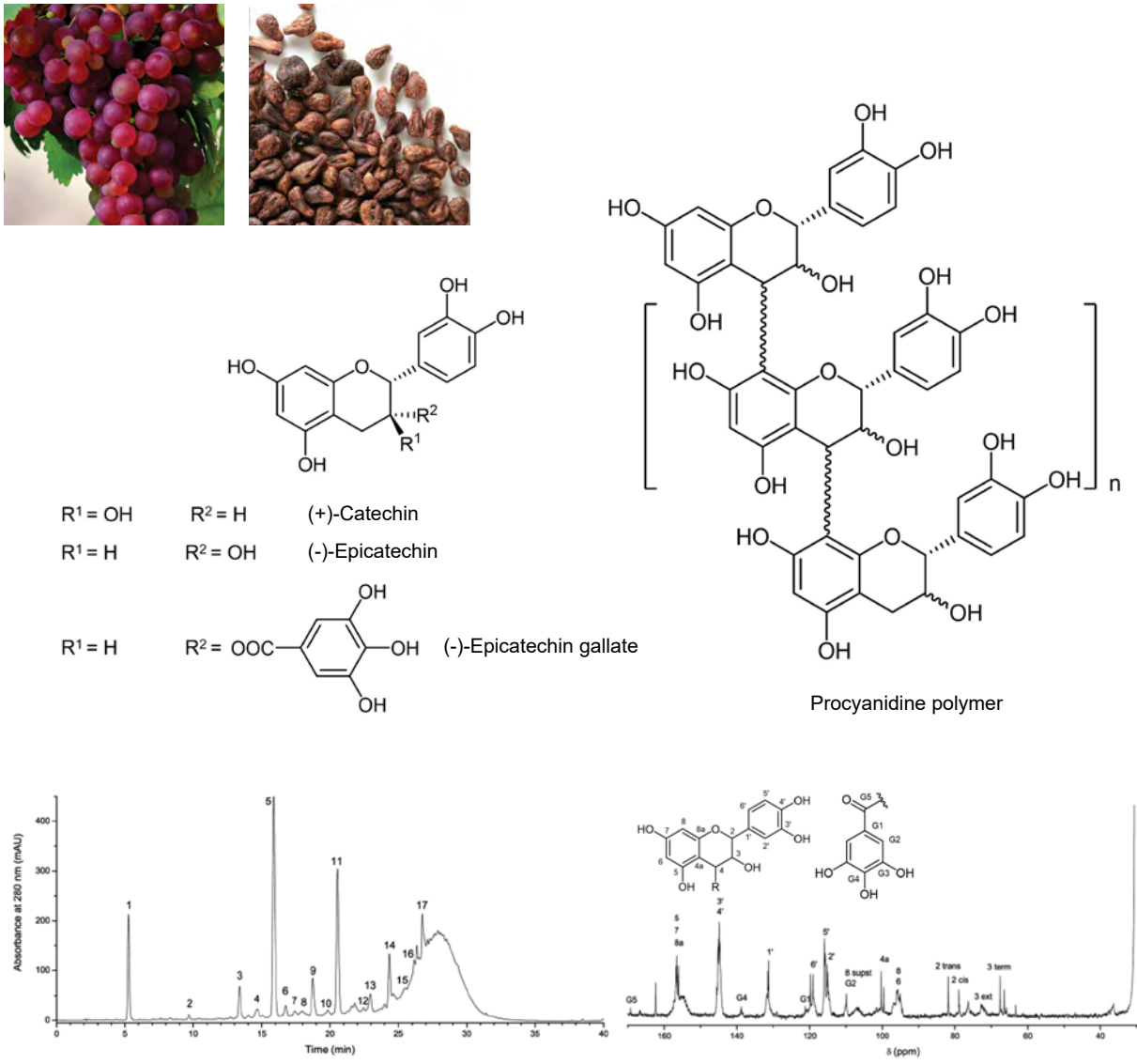


Figure 14. Polyphenols identified on seed surface of grapes Cabernet Sauvignon; left down: Liquid chromatogram (HPLC/DAD) of the extract detected by UV detector and bottom right: ^{13}C NMR spectrum of the same extract.

One of the recent examples from our laboratory is the analysis of the extract (EtOAc/H_2O, 9:1) from the surface of the grape seeds of *Vitis vinifera* L. cv. Cabernet Sauvignon shown in figure 14.

Using different instrumental techniques, such as hyphenated liquid chromatography and mass spectrometry (LC/UV/ESI-ToF MS), nuclear magnetic resonance spectroscopy of carbon-13 (^{13}C NMR), 17 polyphenolic compounds were identified (gallic and protocatechuic acids, catechin and epicatechin monomers, procyanidin oligomers and procyanidin gallates, figure 14). *In vitro* tests revealed considerable efficacy of this extract in the prevention of oxidative lymphocyte damage by ROS.

3.3. The analysis of secondary metabolites of arthropods

3.3.1 Centipedes

The investigation of chemical ecology of arthropods in our laboratory commenced in 2008 in collaboration with the Faculty of Biology, University of Belgrade. The central part in these works belongs to the examination of defensive substances of myriapods belonging to classes Diplopoda and Chilopoda using modern instrumental methods, such as GC/MS, LC/MS, LC/MS/MS, NMR (^{1}H and ^{13}C NMR, COSY, NOESY, DOSY, HSQC, HMBC, ^{15}N HSQC and ^{15}N HMBC), as well as electrophoresis. Our investigations encompassed about one third of all myriapod species inhabiting Serbia. In addition, the species from Montenegro, Croatia, Denmark and Azerbaijan were analysed too. Results revealed a great variety of defensive compounds, involving gaseous compounds such as hydrogen cyanide, as well as aromatic compounds, alkaloids (see figure 16) and even proteins.

Figure 15. Millipede *Pachyiulus hungaricus* (Photo D. Antić).

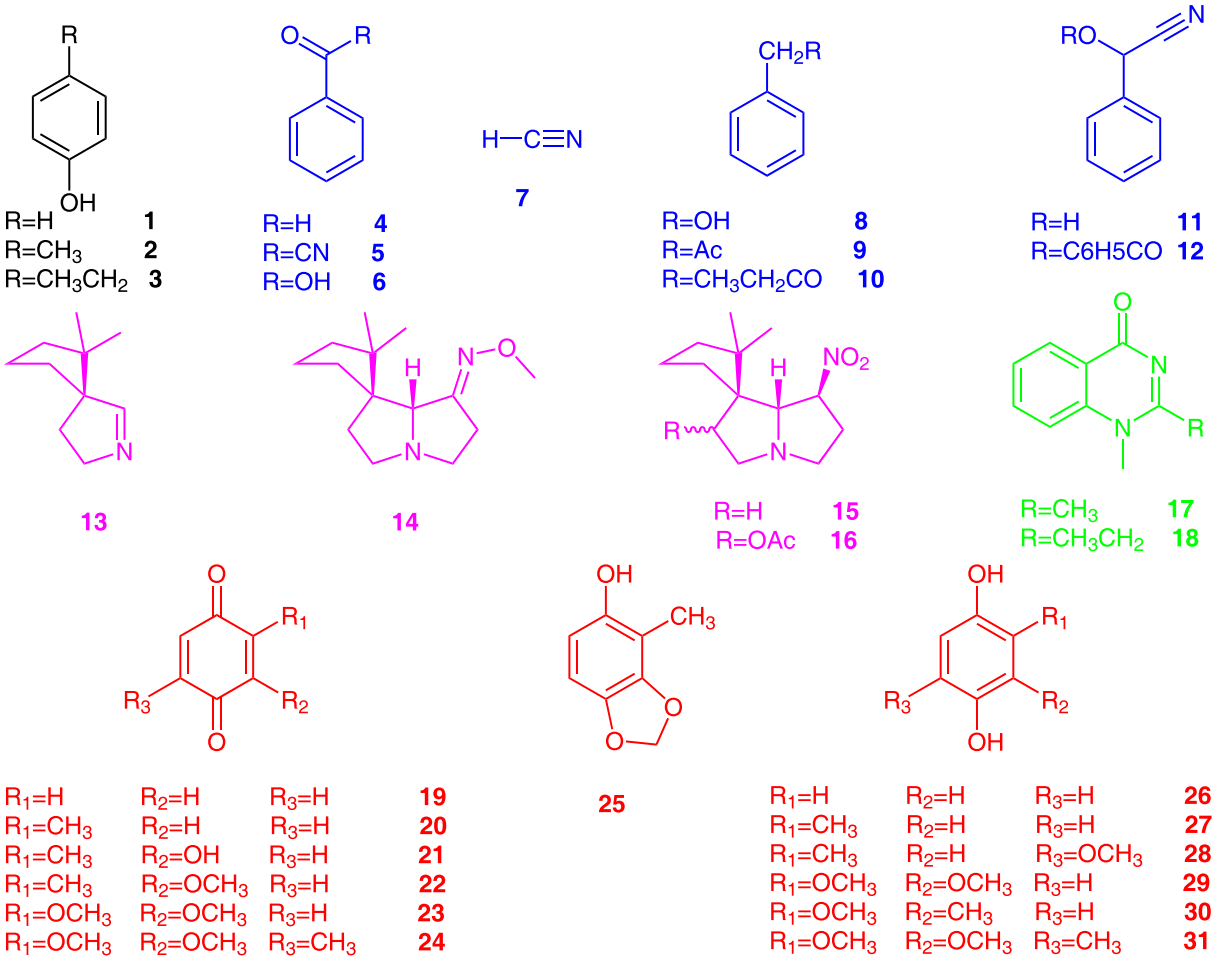


Figure 16. Defensive substances of millipedes.

3.3.2. Insects (beetles/Colleoptera and fruit flies/Drosophila)

In addition to the defensive secretions of centipedes, the defensive substances of beetles from the genus *Carabus* were studied. The volatile carboxyllic acids were the main components of these secretions. In addition to the defensive mechanisms, the study of communication methods between insects is also very important part of our investigations. *Drosophila melanogaster*, the fruit fly was chosen as a model system for this study. After breeding over many years, using different feeding substrates (more than three hundreds of generations of fruit flies feeded exclusively with one sort of substrate: apple, banana, maize, tomato or carrot), the sexual selection was proved by identification of pheromone profiles by means of gas chromatography coupled to mass spectrometry (GC/MS).

4. Miscellaneous analyses

Due to the collaboration with the many different institutions, such as Ministry of Internal Affairs, Municipal Office for Health Protection, Military Medical Academy, pharmaceutical companies etc., a large part of the activities of the Laboratory for Instrumental Analysis comprises expert analyses of illicit drugs, precursors for illicit drugs, (false) medicines, chemical weapons, identifications of stones from the urinary tract etc. These activities are illustrated by a couple of examples in the following text.

4.1. Stones from the urinary tract

Infrared spectroscopy enables rapid and unambiguous identification of the stones excreted from the urinary tract. The Laboratory for Instrumental Analysis, since the foundation in year 1966, has carried out quite a few such analyses. It should be pointed out that they have been accomplished free of charge. Figures 17 – 19 show infrared (IR) spectra of some most common types of stones from the urinary tract measured in our laboratory.

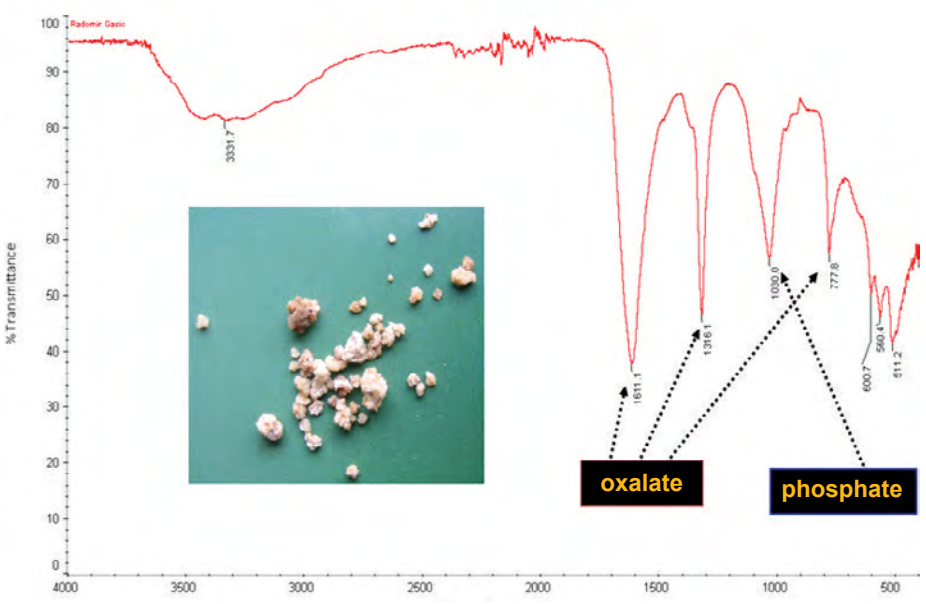


Figure 17. IR spectrum of a “mixed” kidney stone – oxalate + phosphate – excreted by a patient after lithotripsy (breaking up stones).

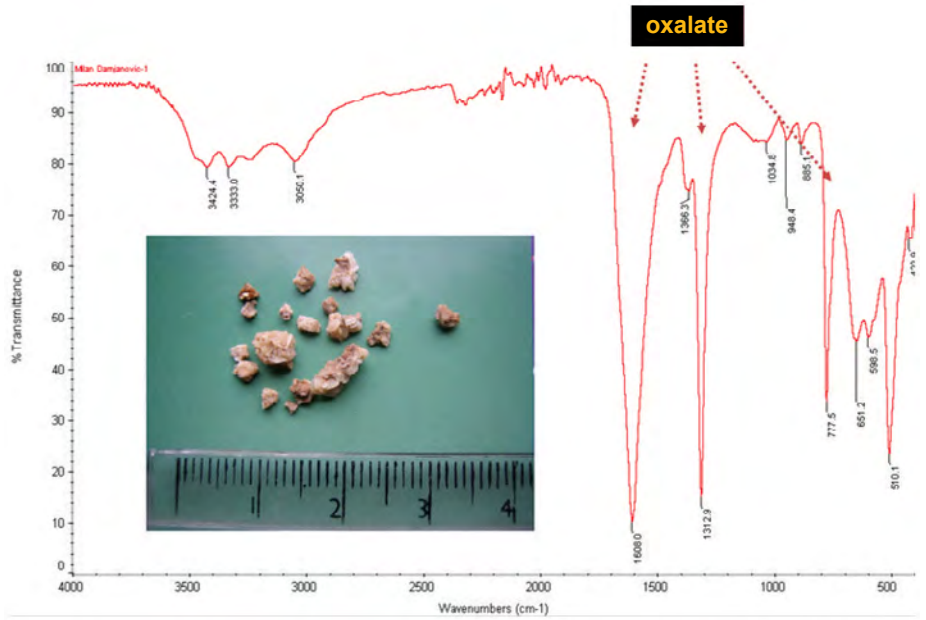


Figure 18. IR spectrum of a kidney stone – oxalate– excreted by patient after lithotripsy (breaking up stones).

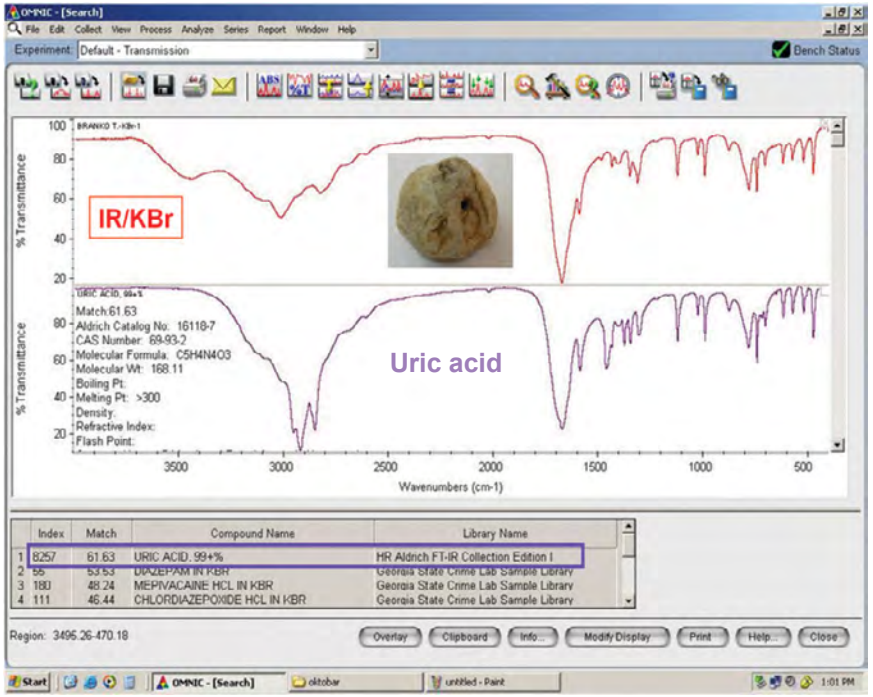


Figure 19. Upper figure: IR spectrum of a stone – ureate – extracted from a gall bladder; lower figure: IR spectrum of uric acid from the data base.

4.2. Precursors of illicit narcotic and psychotropic drugs – acetic acid anhydride

The analysis of an unknown liquid taken from a tank truck held at the border crossing Horgoš was carried out on demand of the Ministry of Health. The content of the tank truck was officially declared as “anhydrous acetic acid”. The NMR and IR spectra of the suspicious sample taken from the tank truck (figure 20) unambiguously revealed acetic acid anhydride, not acetic acid as declared. Obviously, this was an attempt of illegal importation of acetic acid anhydride (AA), the chemical whose traffic is strongly controlled because it is on the list of precursors used for heroin production (by acetylation of morphine) and also for the synthesis of benzyl-methyl ketone, the basic precursor for (Leuckart) synthesis of amphetamine.

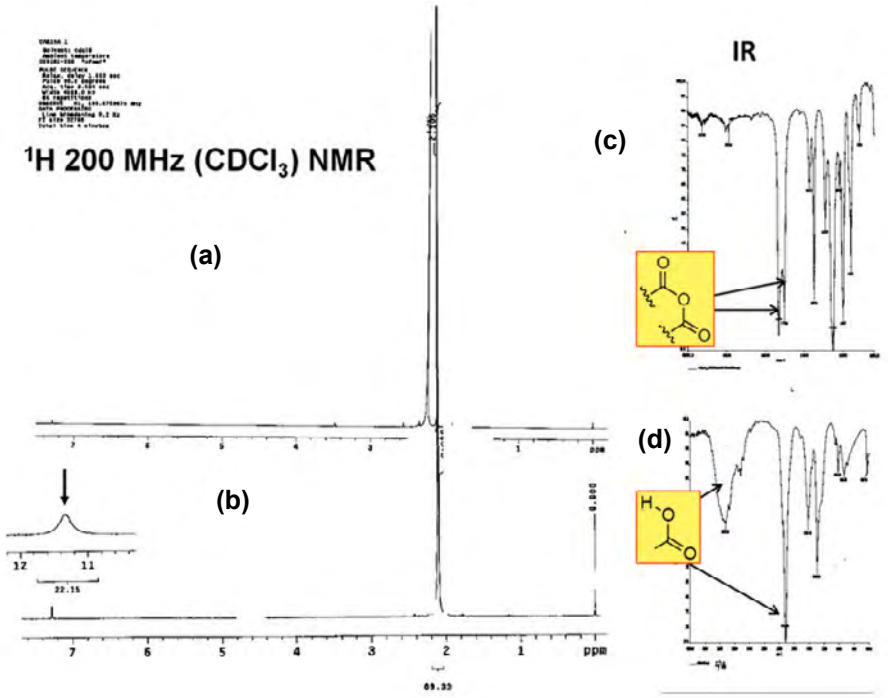


Figure 20. Proton NMR spectra of (a) the sample of "anhydrous acetic acid" taken from the tank truck and (b) glacial acetic acid *p.a.*; IR spectra of (c) the sample of "anhydrous acetic acid" taken from the tank truck and (d) glacial acetic acid *p.a.*

4.3. Illegal production of amphetamine

Our laboratory received *ca.* 100 samples from the Agency for Medicines, collected during action of the police in industrial plant "Lenal pharm", suspected of illegal production of illicit chemical substances. The samples were analysed using infrared (IR) spectroscopy and nuclear magnetic resonance (^{1}H NMR, figure 21).

These spectra unambiguously proved the production of amphetamine. Amphetamine and its N-methyl derivative are among the illicit psychotropic drugs stimulating central nervous system. They induced numerous unwanted effects such as hypertension and long-term addiction leads to violent destructive behavior and acute psychosis similar to paranoid schizophrenia.

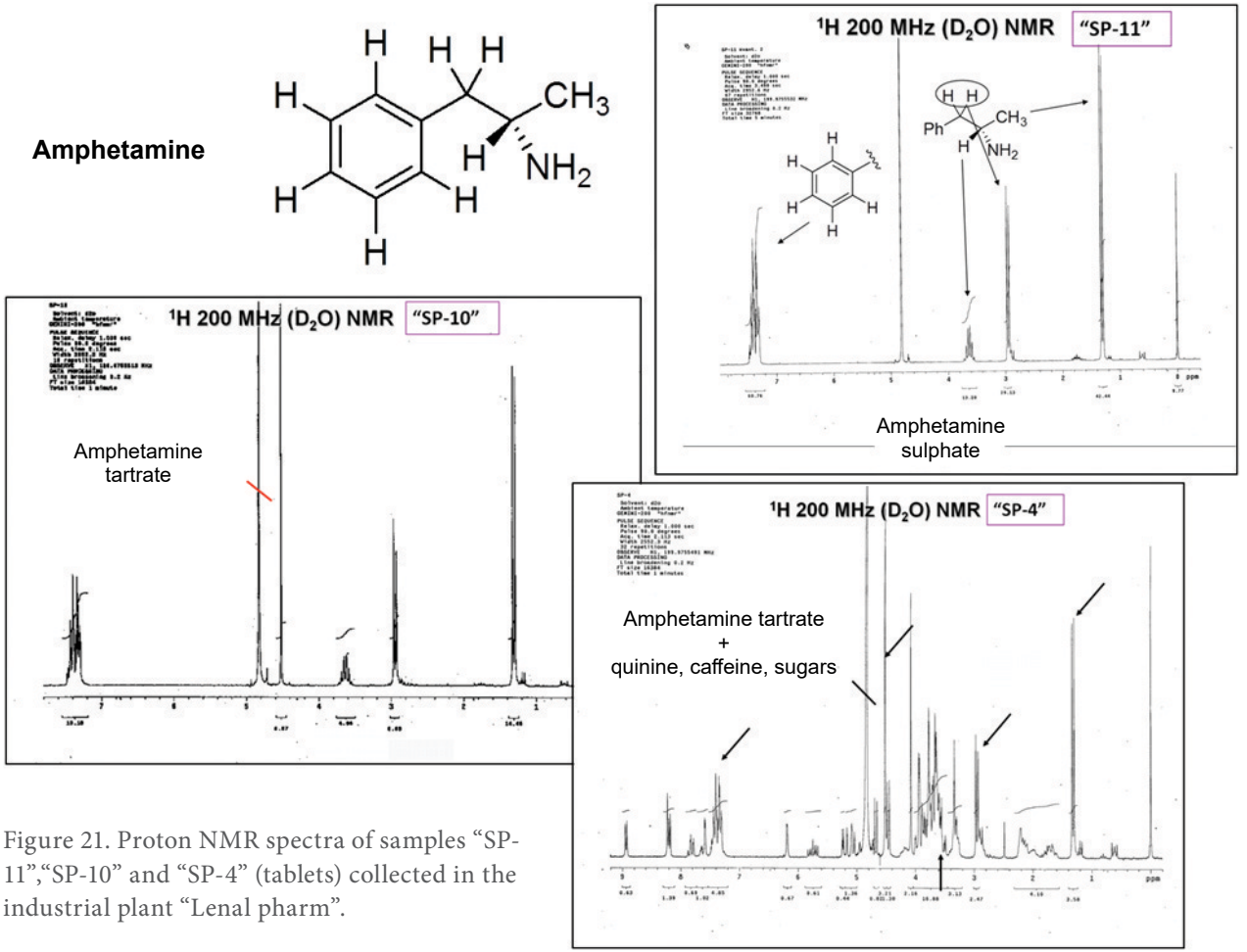


Figure 21. Proton NMR spectra of samples "SP-11","SP-10" and "SP-4" (tablets) collected in the industrial plant "Lenal pharm".

Epilogue: The owner of the firm as well as the remaining participants in this case were sentenced to prison.

4.4. Fake antitetanus vaccine

From the Belgrade Police Department our laboratory received request to carry out analysis of imported antitetanus vaccine ***Tetaglobuline*** (allegedly produced in the Institute Pasteur Mérrieux Connaught). Figure 22 shows NMR spectra of the lyophilized vaccine (a) and that of vaccine ***Tetagam P*** (ZLB Behring production) bought in a pharmacy in the neighbourhood (b). A big difference between their spectra was obvious at the first glance. The spectrum (broad signals) of *Tetagam P* was typical for a protein molecule which should be the active constituent in the correct antitetanus vaccine, whereas the spectrum of the sample under suspicion unambiguously indicated the presence of gentamicin, the broad spectrum antibiotic, *ca.* ten times cheaper than the antitenus vaccine. It also should be noted that gentamicin is not effective against *Clostridium tetani*, the causative agent of tetanus. Thus, it could be concluded that so called ***Tetaglobuline*** was the fake vaccine.

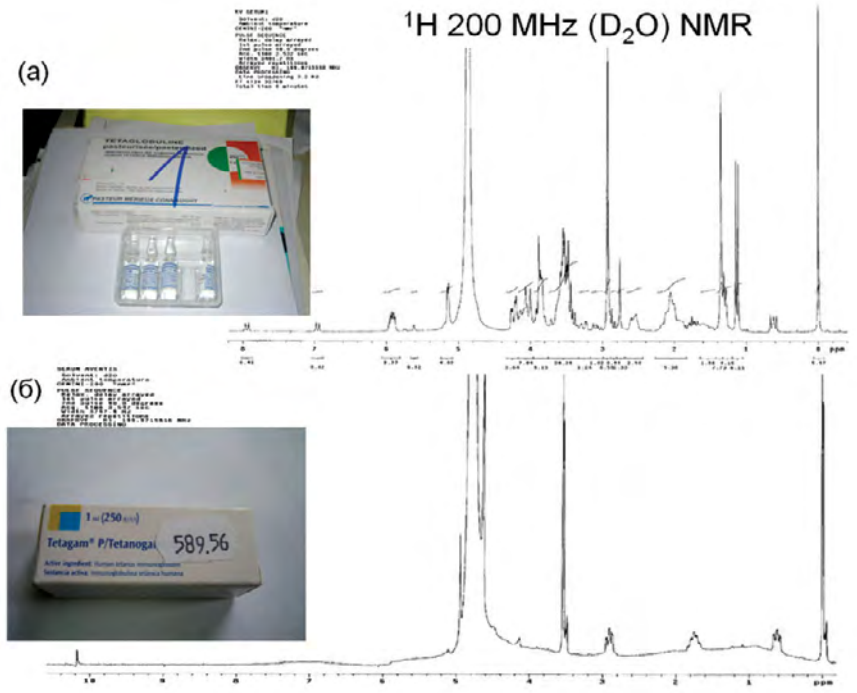


Figure 22. Proton NMR spectra of (a) the fake vaccine imported under the name *Tetaglobuline* and (b) the correct vaccine baught in the pharmacy under the name *Tetagam P.*

4.5. Fake natural herbal supplements - Satibo capsules for treatment of erectile dysfunction

From the Municipal Office for Health Protection we received to analyse the sample of ***Satibo*** (**Fuji Satibo Capsule**) for treatment of erectile dysfunction, declared as 100% natural herbal preparation, based on Chinese traditional medicine (figure 23).

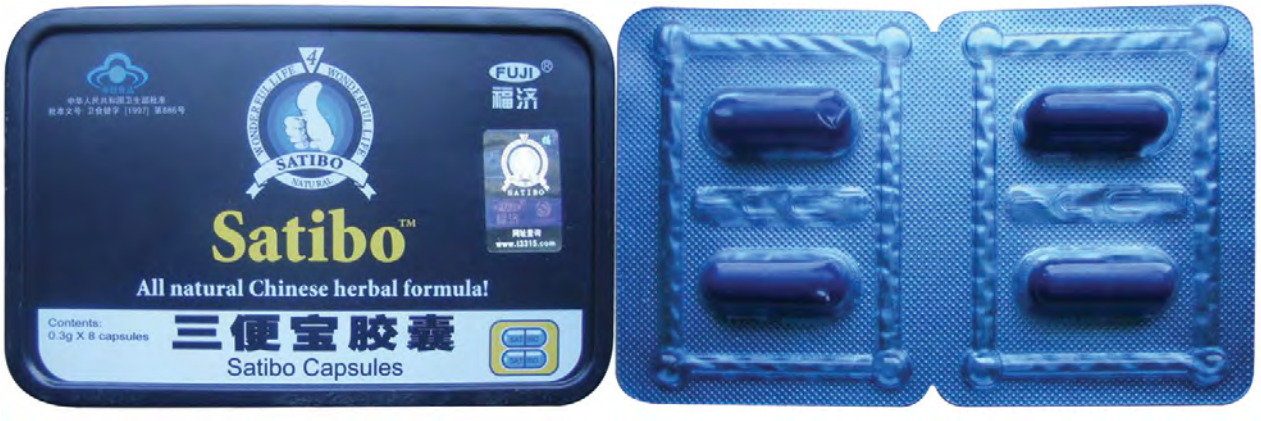


Figure 23. *Satibo* (Fuji Satibo Capsule) capsules for treatment of erectile dysfunction.

By use of hyphenated liquid chromatography/mass spectrometry (LC/UV/ESI-ToF MS) (see figure 23) and two-dimensional NMR spectroscopy (not shown) it was established that the "natural preparation" ***Satibo*** contains synthetic compounds used to treat erectile dysfunction: **Viagra** and **Cialis** (figures 24 and 25).

Thus, it is obvious that the analysed sample was the fake natural preparation.

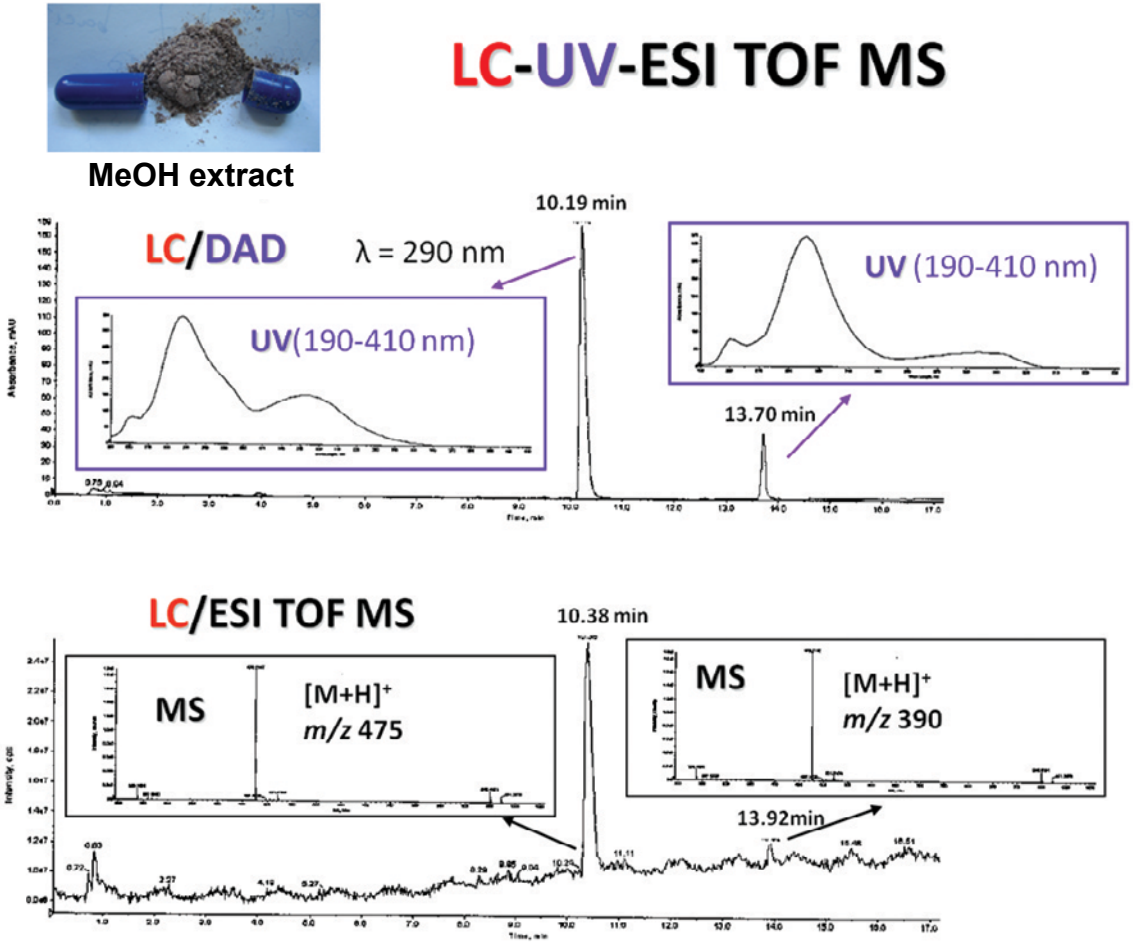


Figure 24. LC/UV/ESI-ToF MS analysis of *Satibo* capsules.

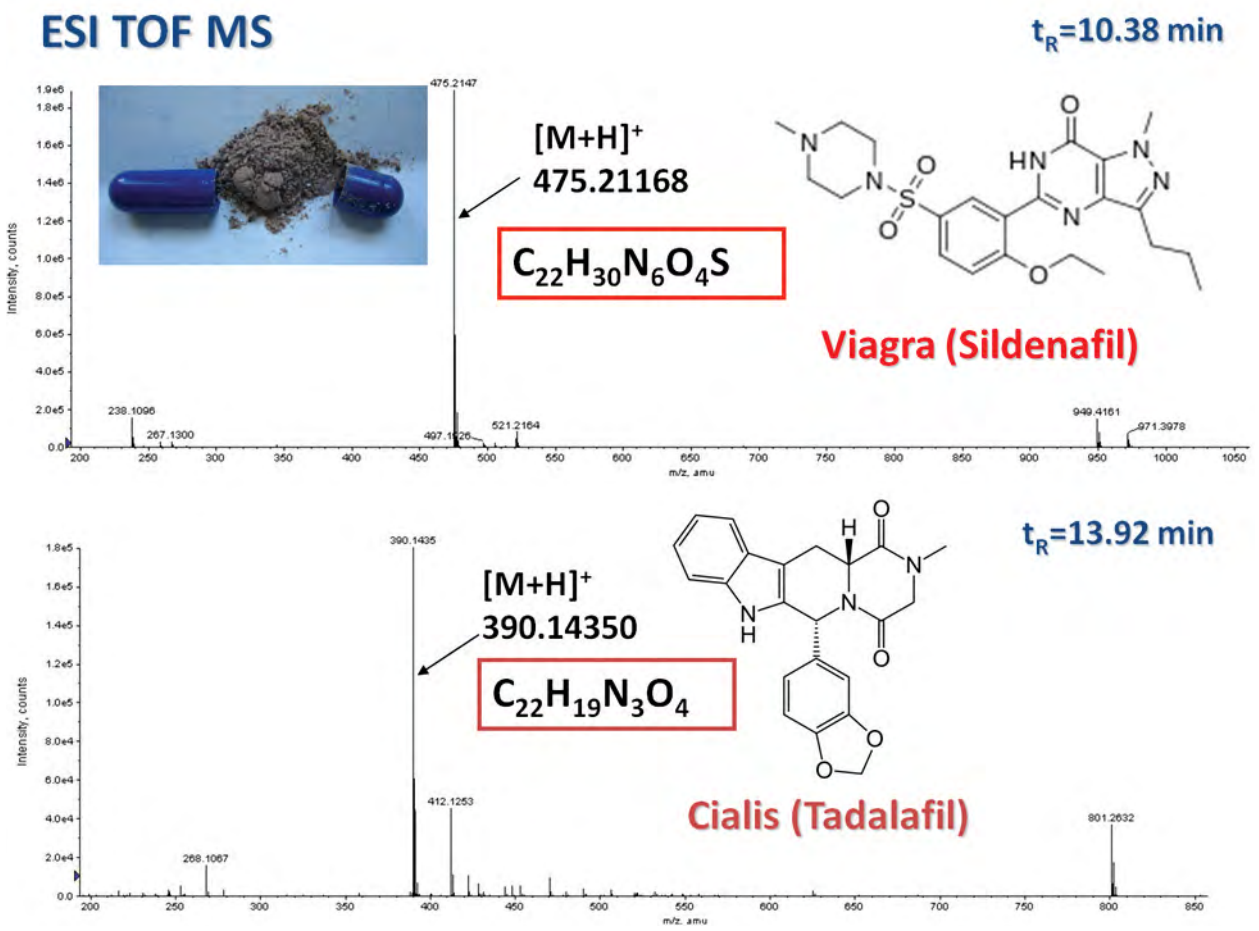


Figure 25. Mass spectra of the constituents of methanolic extract of Satibo capsules indicating synthetic compounds Viagra and Cialis.

5. Proficiency test under the auspices of the Organisation for Prohibition of Chemical Weapons (OPCW)

The succesful results of Proficiency tests in which the Laboratory for Instrumental Analysis participated, carried out under the auspices of the Organisation for Prohibition of Chemical Weapons (OPCW), the latter cooperating with United Nations, located in The Hague, Netherlands demonstrated the high competence of the Laboratory (according to world criteria). This, rather difficult and complicated test is organized in order to find in the participating countries laboratories (so called ***designated laboratories***) competent to reliably analyse toxic substances, able to react promptly in case of the possible accident, *i.e.* application of chemical weapons.

Since 2008 our laboratory has taken part, as the only from this region, with considerable success in the Proficiency tests, organized by **OPCW** twice a year. In course of four tests, involving identification of unknown constituents in very diluted samples containing: toxic substances used as chemical weapons, their degradation products, their precursors and byproducts in their synthesis, all present compounds have been identified (mainly nervous poisons e.g. alkyl phosphonates and blister agents: mustard gases (organosulphur compounds) and Lewisites (organoarsenic compounds).

In the 35th Proficiency test carried out in May 2014, involving 19 participating laboratories from all over the world, laboratories from USA, Switzerland, Romania and our laboratory were rated the highest mark – "A". In that test two more laboratories: Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN), Finland and Defense Chemical Research Laboratory, Islamic Republic of Iran also scored mark A as evaluating laboratory and sample preparation laboratory, respectively. Figure 26 shows compounds identified in the 35th Proficiency test.

For Serbia, as a signatory of the Convention and a member of the Organisation for Prohibition of Chemical Weapons this was a considerable success, taking into account that laboratories from big economically developed countries such as: USA, Russion Federation, China, France, Netherland and Great Britain usually take part in the testing.

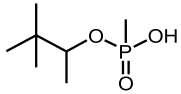
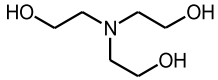
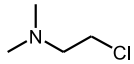
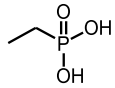
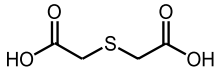
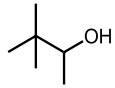
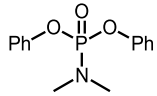
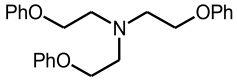
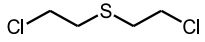
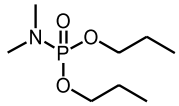
SUMMARY: REPORTED CHEMICALS		
Original Sample Code	Chemical ID assigned by the Laboratory	Chemical name & Structure
351-09	A-1	Pinacolyl methylphosphonate 
351-09	A-2	Triethanolamine 
351-09	A-3	2-(N,N-Dimethylamino)ethylchloride 
353-09	C-1	Ethylphosphonic acid 
353-09	C-2	Thiodiglycolic acid 
354-09	D-1	3,3-Dimethylbutan-2-ol 
354-09	D-2	Diphenyl N,N-dimethylphosphoramidate 
354-09	D-3	Tris(2-phenoxyethyl)amine 
356-09	F-1	Bis(2-chloroethyl)sulfide 
356-09	F-2	Dipropyl N,N-dimethylphosphoramidate 

Figure 26. A page of the official report submitted to OPCW, regarding the Proficiency test carried out in our laboratory in year 2014, showing identfied compounds.

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