

ESA MISSION ROSETTA WILL PROBE FOR CHIRALITY OF COMETARY AMINO ACIDS

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(Received 20 September, 1999)

Abstract. New crucial theoretical investigations on the origin of biomolecular chirality are reviewed briefly. With the goal to investigate these theories our team is going to perform the 'chirality-experiment' in the near future with cometary matter. In 2012 the robotical lander RoLand will detach from the orbiter of the ROSETTA spacecraft and set down on the surface of comet 46P/Wirtanen in order to separate and identify cometary organic compounds via GC-MS *in situ*. Chiral organics will be separated into their enantiomers by application of 3 capillary columns coated with different kinds of stationary phases. Non-volatile compounds like amino acids will be derivatized in especially developed gas phase alkylation steps avoiding reactions in the liquid phase. The results of these preliminary gas phase reactions are presented in this article.

Keywords: amino acid, asymmetric synthesis, chirality, COSAC, cyclodextrin, derivatization, enantiomer separation, gas chromatography, origin of optical activity, Rosetta, 46P/Wirtanen

1. Introduction

1.1. THEORIES FOR THE ORIGIN OF BIOMOLECULE'S HOMOCHIRALITY

Several laboratory experiments generating chirally uniform polymers determined the effect of enantiomeric cross-inhibition (Lundberg and Doty, 1957; Idelson and Blout, 1958; Joyce *et al.*, 1984; Rein, 1992; Brack, 1999). This inhibitory effect gave evidence for the assumption, that life could only arise in an environment disposed of a certain enantiomeric excess. As a consequence of this any questions about the origin of life must deal with *abiogenic* models explaining the origin of the molecular parity violation.

Nowadays various hypotheses receive considerable attention (reviews in Meierhenrich *et al.*, 1999; Thiemann, 1998, 2000): We distinguish between *random mechanisms*, which interpret the appearance of optical activity by a mere chance process (Thiemann, 1974), and the *determinate mechanisms*, which describe the interaction of physical driving forces with racemic substances causing the prevalence of one enantiomer over the other. Extraterrestrial physical driving forces (Khasanov and Gladyshev, 1980) like ultraviolet *circularly polarized synchrotron radiation* emanated at one pole of an extremely rapidly rotating neutron star were suggested



Origins of Life and Evolution of the Biosphere **31**: 199–210, 2001.
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(Rubenstein *et al.*, 1983; Bonner and Rubenstein, 1987; Bonner, 1992) resulting in a topical discussion: Mason (1997) argues against this model, considering the 'Kuhn-Condon zero-sum rule' (Kuhn, 1930; Condon, 1937), that the integral in the circular dichroism (CD) over the whole wavelength range is zero. In case that the neutron star emanates the required radiation over the entire spectrum no enantiomeric enhancement could be produced by photochemical interactions with organic molecules. Very recently Bonner *et al.* (1999) replied that only the CD band of the absorption spectra of prebiotic molecules is relevant for the incriminated enantioselective photochemistry by synchrotron radiation. However, it seems to be difficult to observe and detect *circularly polarized synchrotron radiation* in supernova remnants of pulsars (Bailey *et al.*, 1998; Bailey, 1999).

Apart from this circular polarization of UV-photons caused by Mie scattering on interstellar dust particles in star-formation regions was suggested as a plausible original source for biomolecular handedness. At infrared wavelengths in Orion OMC-1 circularly polarized radiation was indeed recently observed (Bailey *et al.*, 1998).

Earlier the effect of the universal *weak force* (Lee and Yang, 1956) resulting in a theoretically calculated *parity violating energy difference* (PVED) – ultimately responsible for the homochiral biosphere – was taken into consideration by the Yamagata (1966) school (Keszthelyi, 1984; Tranter, 1985; MacDermott and Tranter, 1989; MacDermott, 1993, 1997; Szabó-Nagy and Keszthelyi, 1999).

1.2. THE CHIRALITY EXPERIMENT

With the aim to investigate the specified hypotheses, the European Space Agency is preparing the Cornerstone Mission ROSETTA, to visit the nucleus of the comet 46P/Wirtanen (Jorda and Rickman, 1995; Schulz, 1999) through its robotical Lander RoLand (Ulamec *et al.*, 1997, 1999). Our laboratory participates in the development of ROSETTA's COSAC Experiment (Rosenbauer *et al.*, 1998), that is designed to identify organic molecules in the cometary matter *in situ* (for the chemical composition of comets cf. Kissel *et al.*, 1986a, b; Kissel and Krueger, 1987; Jessberger *et al.*, 1988). The delivery of similar organic compounds as precursor molecules for life to the early Earth in the heavy bombardment phase happens to be astronomically plausible (Oró, 1961; Oró *et al.*, 1971; Oberbeck and Aggarwal, 1992; Huebner and Boice, 1992; Bernstein *et al.*, 1999a, b; Ehrenfreund, 1999a, b).

The 'chirality-experiment' of the COSAC unit is going to identify enantiomers of non-complex organic molecules like alcohols, diols, amines etc. and to determine their enantiomeric enhancement in the matter of the comet's nucleus (Meierhenrich *et al.*, 1999).

We try to separate these enantiomers using the technique of capillary gas chromatography (GC) on the surface of comet Wirtanen at 3 AU with near-zero gravity and negligible gaseous atmosphere. The GC as compared to the liquid chromato-

graphy (HPLC) has several advantages for its use both in space and on the comet's surface: GC offers more theoretical separating plates, the efficiency and sensitivity are higher, the limits of detection lower, the analysis time is shorter as well as the column equilibration time, GC requires no solvent, it is easily coupled to a mass spectrometer (MS), and the selectivities could simply be modified by temperature alone.

1.3. ACCESS TO AMINO ACIDS ANALYSIS

Amino acids play a crucial role in living organisms and possibly in the origin of homochirality (Meierhenrich and Thiemann, 1999). The L-amino acids predominate in molecules produced via biological pathways (e.g. in protein sequences), whereas its optical antipodes, the D-enantiomers, are of negligible occurrence in biological systems. Some exceptions however started to receive considerable attention in the literature (Nagata *et al.*, 1992a, b, 1994, 1998; Kullman *et al.*, 1999).

In case of the COSAC chirality experiment we have to consider that amino acids are too polar to get access to their analysis directly by gas chromatography. The volatility of these intramolecular zwitterionic compounds appears to be too low. To illustrate this effect a solution of the amino acid D,L-valine in methyl alcohol was injected into a gas chromatograph. The resulting chromatogram is given in Figure 2 insert. With the exception of the solvent peak (methyl alcohol, $t_R = 1.0$ min) no compound was eluted.

Compared with the parent amino acids, esters are non-polar, much more volatile, and do chromatograph well. Esterification is therefore the obvious first choice for a derivatization of amino acids, particularly for the intended gas chromatography on the comet's nucleus. Conventional methods of esterification include reaction with diazomethane or higher diazoalkanes or esterification with methanol-acid mixtures. Diazomethane is unstable, toxic and explosive, and the transesterification step requires solution chemistry. These properties exclude these conventional methods from our project. There was thus a need for a reliable working 'dry' method for the preparation of amino acid esters on COSAC's GC. We have therefore developed and optimised a simple and 'dry' derivatization technique in which amino acids are transformed into their dialkylamino alkyl esters by a gas phase reaction step within the injection port of the gas chromatograph itself.

Two methods for such a gas phase derivatization technique were evaluated: The pyrolysis of quarternary ammonium salts and the use of alkyl substituted acetals as reagent.

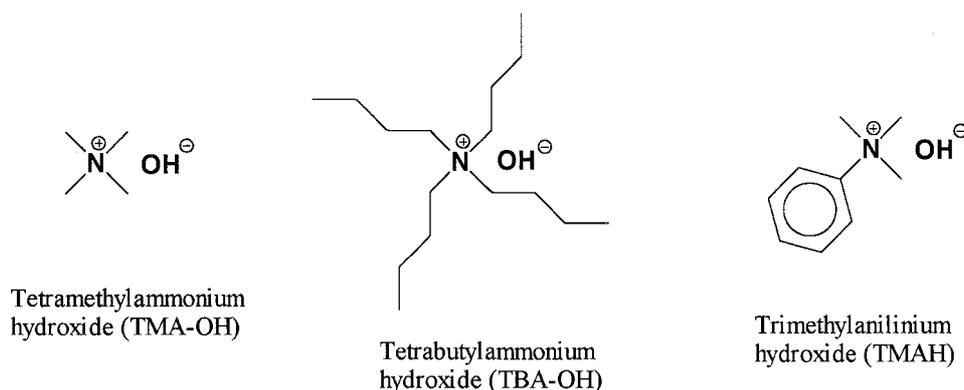


Figure 1. Chemical formulae of pyrolysis assisting quaternary ammonium salts.

2. Experimental

2.1. PYROLYSIS ASSISTING QUARTERNARY AMMONIUM SALTS

It has been known since the early experiments of Robb and Westbrook (1963) that acids are transformed into their methyl esters if they are heated in the presence of specific quaternary ammonium salts like tetramethylammonium hydroxide (TMA-OH, cf. Figure 1). This pyrolysis technique coupled with gas chromatography (Py-GC) was applied successfully in the identification of hydrogen cyanide polymers, too (Minard *et al.*, 1998). The pyrolysis reaction requires temperatures of 365 °C in the heated injection port of a gas chromatograph; this temperature is too high for the intended application on the comet's surface. A further disadvantage of this method is that the analytes were titrated against TMA-OH prior to Py-GC. In order to improve this technique for formic and lactic acid, the corresponding tetrabutylammonium hydroxide (TBA-OH, cf. Figure 1) was synthesised (Schwarze and Gilmour, 1969) and applied in an analog way. Subsequently the trimethylanilinium hydroxide (TMAH, cf. Figure 1) was introduced (Middleditch and Desiderio, 1972) in preference to the corresponding methyl and butyl compounds; it had been widely used as a methylating agent for a variety of different classes of compounds (Barnes *et al.*, 1982).

The elegance of the Py-GC technique is, that the whole derivatization procedure was achieved within the injector system of the gas chromatograph: Initially, 2 μ L of the liquid quaternary ammonium salt were injected together with an 0.2 to 1.0 μ L solution of the acid to be analysed in methyl alcohol. Below 200 °C no derivatives, partial or permethylated, were detected. Whilst the monohydroxy acids were converted at 250 °C, maximum Py-GC conversion did not occur until 350 °C (cf. Meierhenrich *et al.*, 2000). This temperature range is – as mentioned above – not applicable for the intended Py-GC technique on the comet's surface, which demonstrates the need for reliable reagents working at lower temperatures.

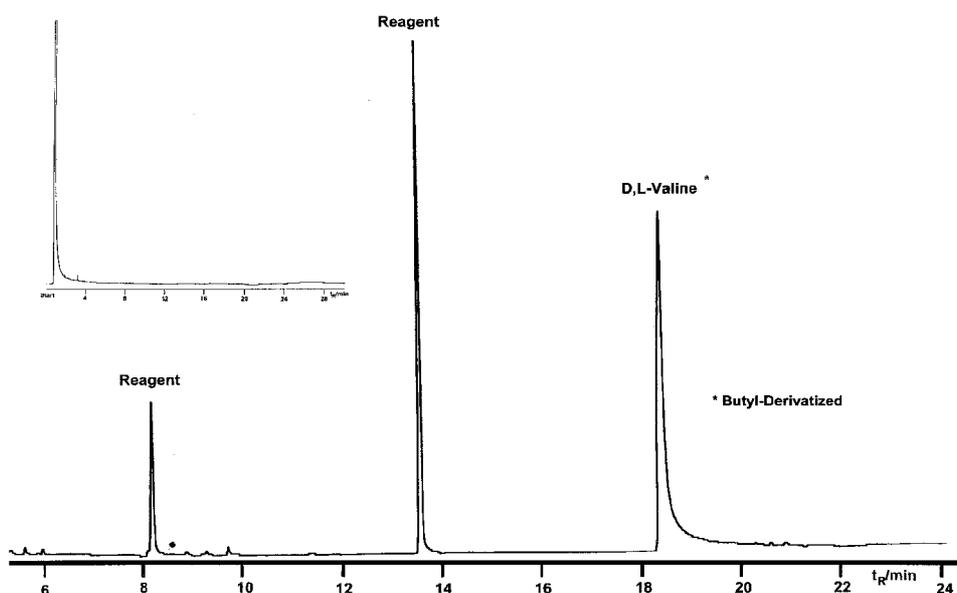


Figure 2. Chromatogram of gas phase derivatized D,L-valine. Insert: blank-chromatogram of D,L-valine without reagent.

2.2. PYROLYSIS ASSISTING DIMETHYLFORMAMIDE DIALKYLACETALS

Alkyl substituted acetals are more suitable reagents, which could be used to esterify acids into their alkyl esters by gas phase reactions using the Py-GC technique (Thenot *et al.*, 1972; Thenot and Horning, 1972; Chiang, 1980). Methyl, ethyl, *n*-propyl, *n*-butyl and *t*-butyl esters could be produced analogously: The acid sample was – similarly to the quarternary ammonium salt procedure – either dissolved in methyl alcohol and reacted with a liquid acetal or reacted directly with the liquid alkyl substituted acetal dissolved therein without any additional solvent. The educts were directly injected into the heated injection port of the gas chromatograph, where the dialkylamino alkyl esters were produced by the gas phase reaction, and swept directly onto the capillary column.

For the experimental work on this study a 0.5 to 1 μL aliquot of the appropriate solution of the amino acid in methyl alcohol (5 to 10 mg L^{-1}) together with 0.2 to 0.5 μL of the pure reagent (dimethylformamide dialkylacetal, purchased from Fluka Chemica, cf. chemical formulae in Figure 3) was subjected by split 1:100 injection at 200 °C and carrier gas hydrogen (pressure: 90 kPa) into a gas chromatograph's injector port (Perkin Elmer 8500 equipped with a Shimadzu Chromatopac C-R6A Integrator). A 25 m Chirasil-D-Val stationary phase (I.D.: 0.25 mm, film thickness: 0.08 μm) was used for enantioselective chromatography using optimized oven temperature profiles; the obtained compounds were detected by a flame ionization detector (FID).

3. Results

The feasibility of the DMF-DBA assisted Py-GC/FID technique for amino acids is demonstrated in Figure 2. Racemic D,L-valine was perbutylated under optimum pyrolysis conditions (oven temperature profile: 4 min 70 °C, 5 °C min⁻¹, 5 min 175 °C; reagent: 0.2 μL dimethylformamide dibutylacetal [DMF-DBA]). The gas phase synthesis of the volatile *N,N*-dibutyl-2-amino-3-methylbutanoic acid butylester (i.e. perbutylated valine, $t_R = 18.52$ min) appears to be quantitative, reproducible and is easily to handle. Additional peaks of DMF-DBA side-products, eluted at considerably smaller retention times of 8.28 and 13.66 min, were not interfering with the amino acid's analysis. The enantiomers however could not be separated from each other with the here chosen stationary phase. The chemical equation in Figure 3 exemplifies the acetal assisted gas-phase pyrolysis reaction.

The obtained Py-GC/FID enantiomer separation of D and L-phenylalanine is depicted in Figure 4. The analysis system was optimized with respect to oven temperature (isotherm 140 °C) and pyrolysis assisting reagent (0.2 μL dimethylformamide dimethylacetal [DMF-DMA]). Based on these improvements a separation factor $\alpha = 1.09$ and a resolution $R_S = 3.75$ was achieved.

Hence the 'dry' and simply handled acetal assisted Py-GC technique will be applied within COSAC's gas chromatograph, to transform polar organic compounds like amino acids into products of excellent gas chromatographic properties. The ability to perform rapid and routine analysis of several non-volatile organic compounds makes Py-GC/FID an attractive technique to determine polar chiral organics in any available extraterrestrial matter, e.g. in meteorites. It will be systematically evaluated on a number of amino acids in the near future. The analogue feasibility of the Py-GC/FID technique on chiral carboxylic acids was recently reported by Meierhenrich *et al.* (2000).

4. Discussion

One would expect far-reaching results from a discovery of enantiomer compounds on comet 46P/Wirtanen by ESA's robotical lander RoLand (Figure 5).

If the observed molecular parity violation on Earth were mainly caused by *random mechanisms* one would naturally expect to find enantiomers of different handedness on different planetary or interstellar bodies. If homochirality were however determined by *circularly polarized radiation* from a passing neutron star all planets within a given solar system would have been affected by the same neutron star's pole and their biomolecules would be produced stereospecifically in the same configuration. If biomolecular chiral symmetry breaking was originated by multiple (Mie) scattering in star formation regions, these scattering processes would presumably produce antipodic circularly polarized UV-photons as a function of time and location. Therefore one would expect to detect variable enantiomeric

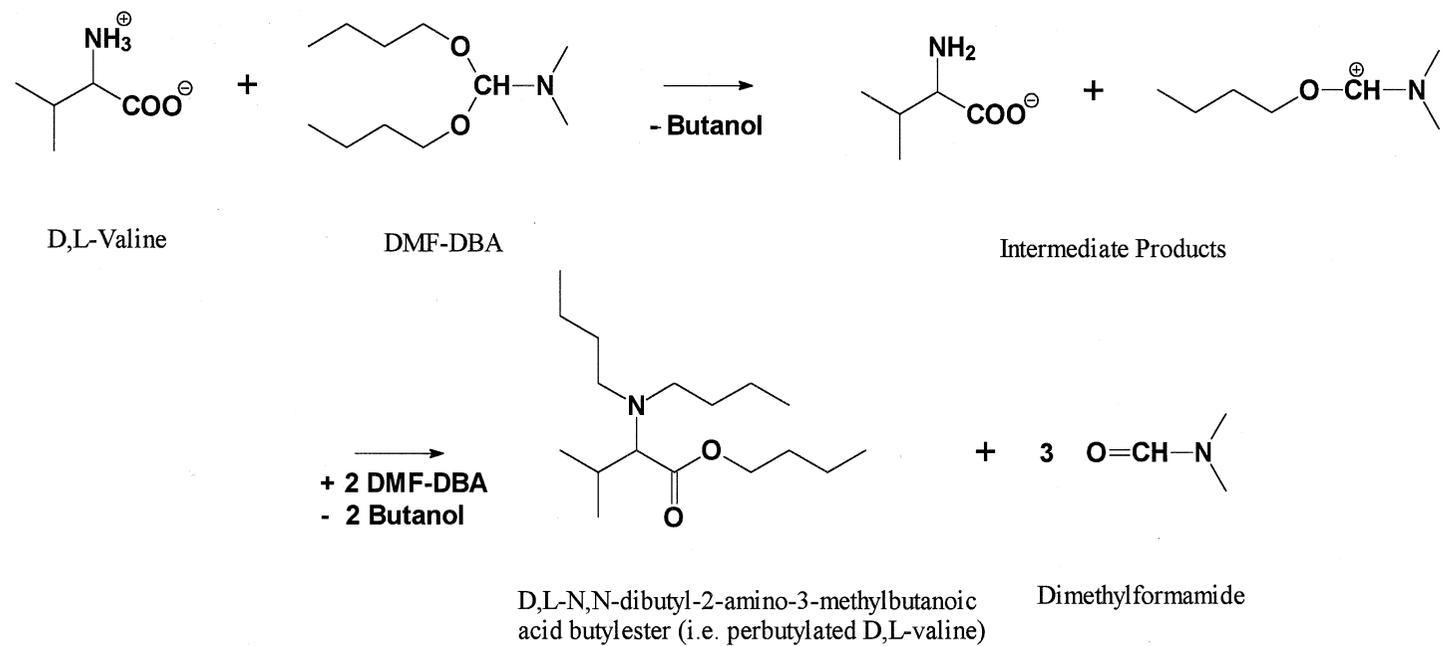


Figure 3. Chemical equation depicting the pyrolysis of DMF-DBA perbutylating non-volatile amino acids.

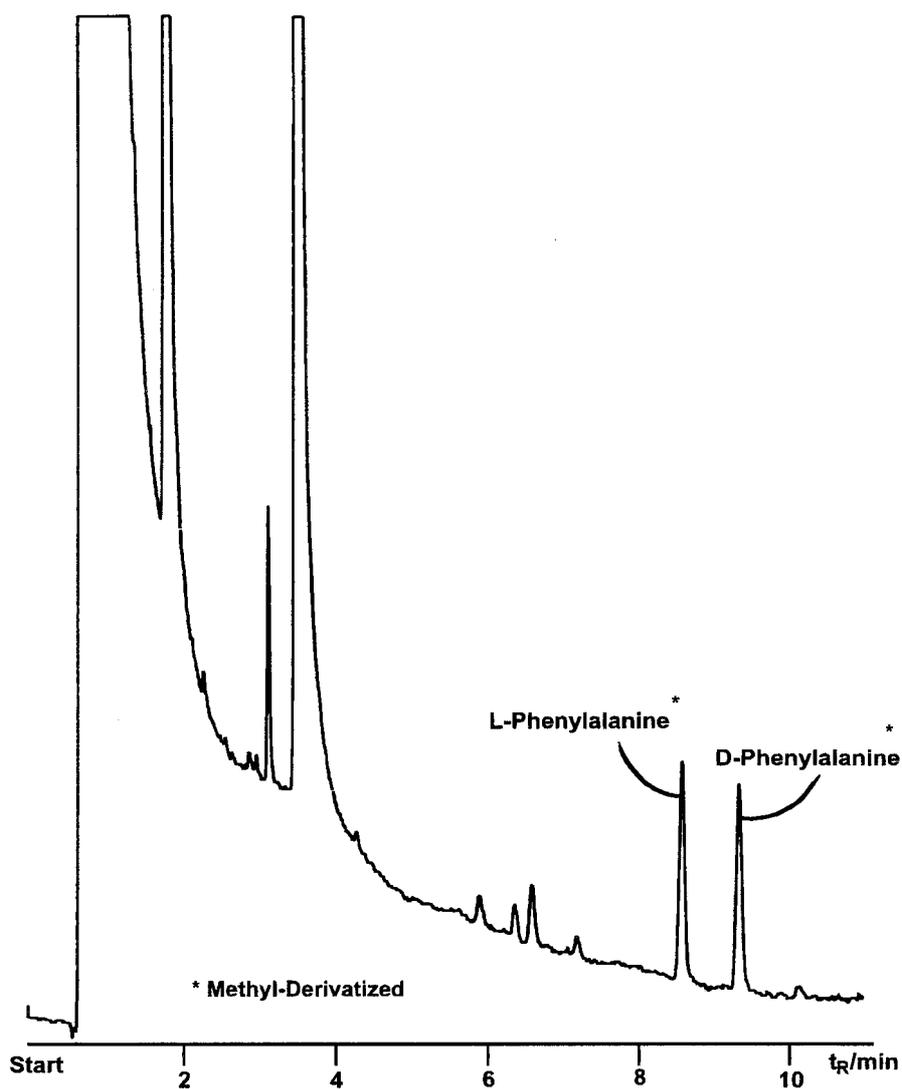


Figure 4. Chromatographic separation of gas phase derivatized D,L-phenylalanine into its enantiomers.

excesses on different planetary or interstellar bodies. And if biology's optical activity was determined by the universal chiral influence of the *weak force*, one would expect to find identical handedness of enantiomers throughout the entire universe.

Acknowledgements

We wish to thank the COSAC-team composed of Jean-Francis Brun, David Coscia, Pascale Ehrenfreund, Fred Goesmann, J. Mayo Greenberg, Guy Israel, Laurent

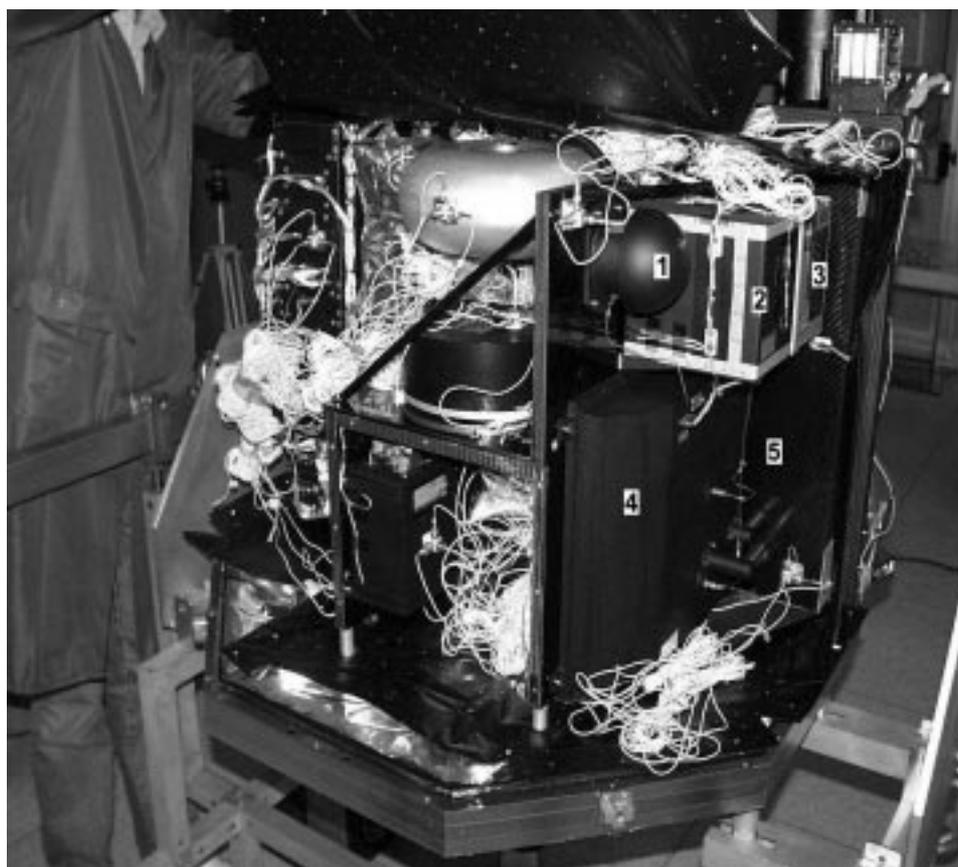


Figure 5. ROSETTA's robotic lander RoLand contains the COSAC Experiment. (1) Primary Helium Gas Tank, (2) Mass Spectrometer E-Box, (3) Gas Chromatograph, (4) Mass Spectrometer, and (5) Secondary Helium Gas Tank. Photo: DLR/ESA.

Janin, Elmar K. Jessberger, Alexandra J. MacDermott, T. Matsuo, Guillermo Munoz-Caro, Francois Raulin, Reinhard Roll, Helmut Rosenbauer (PI*), Robert Sternberg, Cyril Szopa, and Sandine Zubrzycki for their scientific support. U.M. thanks the sponsors of the Max-Planck-Institut für Aeronomie (MPAe) for a post-doc stipendium and the Deutsche Forschungsgemeinschaft for the ISSOL travel grant.
* Principal Investigator.

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