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Short Communication

Use of mass spectrometry for the detection and identification of bromine-containing diphenyl ethers

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ABSTRACT

The gas chromatographic–resonance electron-capture mass spectrometric determination and identification of physiologically active bromine-containing compounds in the products of metabolic activity of marine symbiotic microorganisms are described. It is shown that the bacteria *Vibrio* sp. associated with the sponge *Dysidea* sp. are capable of producing brominated diphenyl esters.

INTRODUCTION

In recent years, interest in searching for new producers of physiologically active substances from microorganism has been increasing [1–3]. For a successful search for such microorganisms, it is necessary to use sensitive analytical techniques that permit the determination of small amounts (sometimes picomoles) of the necessary metabolites.

The combination of chromatography with mass spectrometry (MS) is the most reliable and sensitive technique. The use of specific detectors allows the separative capability of chromatography to be increased and an enhanced analytical resolution in the case of a poorly purified mixtures. This can be achieved by using element-selective, structure- or functional selective or property-selective detectors [4]. This applies to gas chromatography (GC)–MS. For example, if substances studied possess a high electron affinity, the use of GC–negative ion chemical ionization (NICI) MS increases the sensitivity even more.

In addition to these widely applicable methods, MS with resonance electron capture ionization (REC-MS) has been developed. In comparison with the traditional MS (including GC–MS), its characteristic feature is a greater specifity due to the energetic selectivity of ionization (the resonance character of ionization) [5]. So far REC-MS has not been used for the analysis of bioorganic compounds. In this work, REC-MS was used for the detection and identification of physiologically active bromine-containing compounds in the products of metabolic activity of the bacteria *Vibrio* sp. associated with the sponge *Dysidea* sp.

EXPERIMENTAL

Chemical and microbiological studies

Eight strains of microorganisms were isolated from microbiological samples taken from two collections (Tuluifa and Ofu, East Samoa) of the sponge *Dysidea* sp., a well known source of 3,5-dibromo-2-(3′,5′-dibromo-2′-metoxyphenoxy)phe-

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nol (TBDPE) [6]. The microorganisms were cultivated on a medium containing peptone (5 g), yeast extract (2.5 g), $MgSO_4 \cdot 7H_2O$ (0.1 g) and sea water (1 l) at 30°C for 72 h.

The culture liquid (1 l) was extracted three times with 150-ml portions of *n*-butanol. The evaporated butanol extract was separated on a silica gel (KSK) column with chloroform–ethyl acetate (20:1). The fractions obtained were tested for brominated compounds by NI-MS [electron impact (EI) 70 V, ionization by secondary electrons]. The bromine-containing fractions were combined and purified on a Sephadex LH-20 column with chloroform–ethanol (7:1), followed by UV detection at 265 nm. One of the 21 fractions obtained showed the presence of brominated compounds (monitoring by NI-MS with EI, 70 V). It was further separated using high-performance liquid chromatography (HPLC) on a Beckman–Altex Si 100 Polyol column (Serva).

Mass spectrometry

MS was carried out on an LKB 2091 mass spectrometer (able to operate in both positive and negative ion modes), combined with a Packard Model 438A gas chromatograph by the use of standard jet-type separator. For working in the REC mode, the filament power chain was modified such that the filament current stabilization could be turned off and the emission current arranged manually. GC was carried out on an SE-54 capillary column (25 m × 0.25 mm I.D.) using a solventless injection system. Helium was used as the carrier gas at a flow-rate of 2.0 ml/min. The temperature of column was increased from 200 to 300°C at 8°C/min. The temperature of the ion source, injector and separator was 250°C. With direct inlet of samples for successive sublimation of mixture components, the temperature of the direct inlet evaporator was increased from 50 to 150°C at 10°C/min. In the experiments in the EI mode, the ionization voltage was set at 70 V (for both positive and negative ions), and in the REC mode at about 4 V (which gave the maximum ion current yield of standard TBDPE isolated from an ethanol extract of the sponge *Dysidea* sp. [6]). Mass spectra were recorded with a UV oscillographic recorder. The instrumental conditions were emission current 25 μA and pressure in the analyser $1 \cdot 10^{-7}$ Torr.

RESULTS

The investigation of standard TBDPE showed that 65% of the total ion current is associated with the bromine ions of *m/z* 79 and 81 (Fig. 1) in the negative ion spectrum (EI, 70 V). We used this fact to detect the bromine-containing compounds in butanol extracts of culture liquids of eight bacterial strains isolated from the symbiotic complex of the sponge *Dysidea* sp. The analysis allowed us to select three strains of microorganisms in whose products characteristic ions with *m/z* 79 and 81 were found. One of the strains, defined as *Vibrio* sp., was used for cultivation in order to isolate the bromine-containing compounds. Successive purification of a butanol extract of culture liquid on Sephadex LH-20 silica gel and HPLC resulted in a bromine-contain-

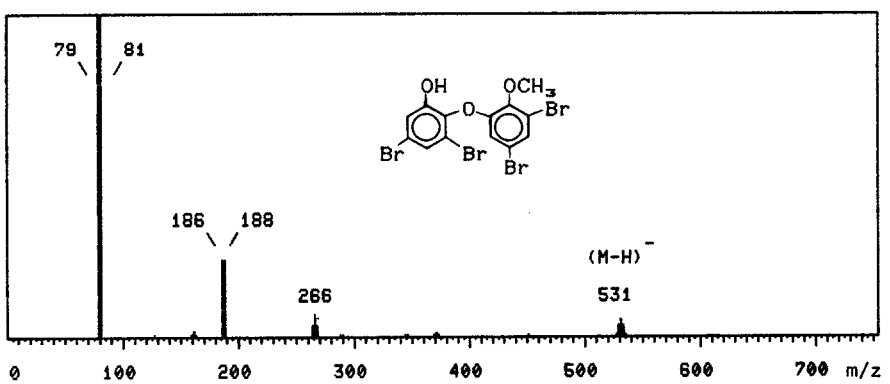

Fig. 1. Negative ion mass spectrum (EI, 70 V) of the tetrabrominated diphenyl ether TBDPE.

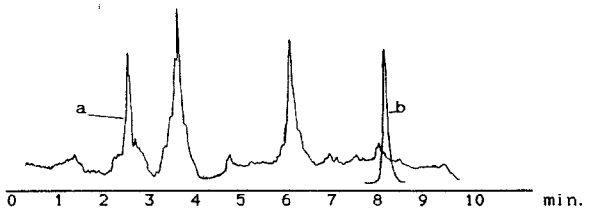

Fig. 2. Chromatograms recorded in a positive ion GC–MS run for (a) a purified butanol extract of culture liquid and (b) for standard TBDPE.

ing fraction (all the steps of the purification were monitored via the bromine ions of m/z 79 and 81 by NI-MS with EI, 70 V). GC showed the presence of a series of compounds (Fig. 2a), among which we failed to identify TBDPE by its retention time (Fig. 2b).

In this connection an attempt to take advantage of the energetic selectivity of REC-MS was undertaken. For this TBDPE was inserted into the ion source through a direct inlet system, and the energy of capture (of resonance) that provides the maximum yield of the total ion current was set. Then GC–MS analysis was carried out at the energy set in this way. As expected, the energetic selectivity provided an almost complete absence of ion current from compounds of other classes and allowed only two peaks to be recorded on the chromatogram (Fig. 3a). In addition to conformity with energies, the first peak also coincided with the standard according to its retention time (Fig. 3b). This allowed us to identify this peak as TBDPE. The retention time and the ionization energy of the second peak allowed it to be attributed to the same type of compound as TBDPE. The REC mass spectra recorded

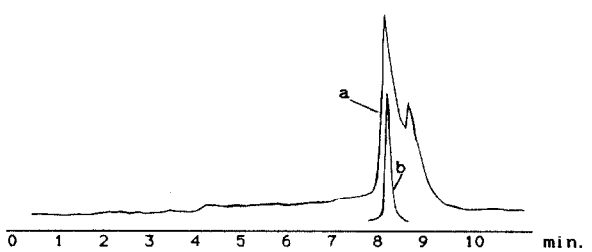

Fig. 3. Chromatograms recorded in a resonance electron-capture GC–MS run for (a) a purified butanol extract of culture liquid and (b) for standard TBDPE.

at this energy are hardly informative, because of up to 65% of the total ion current is associated with the bromine ions. However, knowing exactly the presence of brominated compounds in the sample and their retention times, we could carry out the GC–MS analysis in the positive ion mode and record both compounds. The multiplicity and relative intensities of three peak groups in the high-mass region of the spectrum of the first compound [m/z (%): 536 (16), 534 (65), 532 (100), 530 (70), 528 (18), 440 (14), 438 (42), 436 (43), 434 (15), 374 (9), 372 (17) and 370 (8)] coincided with the spectrum of TBDPE (it was impossible to compare the low-mass region owing to the high level of noise). The spectrum of the second compound established that its molecular mass is 72 u higher than that of TBDPE, and the multiplicity of the molecular ion region indicated the presence of four bromine atoms in this substance also. This result confirmed the inferences which were made above on the basis of the REC-MS data.

Hence the described technique allowed the removal of chemical noise and the identification of brominated diphenyl ethers in the culture liquid of the strain *Vibrio* sp. at concentrations not higher than 3–6 μl/l. Such an approach, with identification by retention time, energy of capture and masses, may be expected to be effective in the search for physiologically active substances in complex mixtures.

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REFERENCES

1 T. Kosuge, K. Tsuji, K. Hirai and T. Fukuyama, *Chem. Pharm. Bull.*, 33 (1985) 3059.
2 M. Yotsu, T.Yamazaki, Y. Meguro, A. Endo, M. Murata, H. Naoko and T. Yasumoto, *Toxicon*, 25 (1987) 225.
3 A. C. Stierle, J. H. Cardellina and F. L. Singleton, *Experientia*, 44 (1988) 1021.
4 P. C. Uden, Y. Yoo, T. Wang and Z. Cheng, *J. Chromatogr.*, 468 (1989) 319.
5 T. Oster, A. Kuhn and E. Illenberger, *Int. J. Mass Spectrom. Ion Processes*, 89 (1989) 1.
6 N. I. Utkina, M. V. Kazantseva and V. A. Denisenko, *Khim. Prirod. Soedin.*, 4 (1987) 603.