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Short Communication

Rapid method for the detection and determination of artemisinin by gas chromatography

A. T. SIPAHIMALANI*, D. P. FULZELE and M. R. HEBLE
Bio-Organic Division, Plant Biotechnology Section, Bhabha Atomic Research Centre, Trombay, Bombay 400 085 (India)
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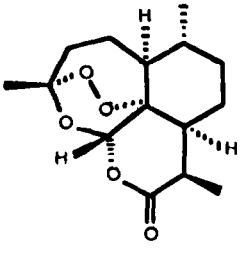
ABSTRACT

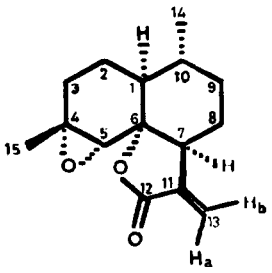
Artemisinin, an antimalarial principle of *Artemisia annua*, is thermally unstable and decomposes on the column to give two peaks under various gas chromatographic conditions. A simple and rapid method was developed for its indirect detection and determination, at nanogram levels, both in plants and in tissue cultures developed from the plants. It is based on the linear relationship obtained between the concentration of artemisinin and the respective peak areas for either of the two thermally degraded products.

INTRODUCTION

Artemisinin (Qinghaosu, QHS) is a naturally occurring antimalarial principle isolated from *Artemisia annua* L. (Compositae) [1]. This compound has attracted great attention because it can offer effective control, against the strains of *Plasmodium falciparum* that have, over the years, developed resistance to chloroquin treatment and also against cerebral malaria in humans [2,3]. *A. annua* is native to China, but in recent years it has been successfully cultivated in the U.S.A., Europe and India. Many



Artemisinin



Arteannuin-B

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laboratories are engaged in developing tissue culture technology [4] for the production of the active principle. Hence it is necessary to develop a suitable technique for its detection and determination at micro- or nanogram levels in both plants and tissue cultures.

High-performance liquid chromatography (HPLC) for the detection and determination of artemisinin requires the use of an electrochemical detector [5], which is not commonly available in many laboratories. The alternative choice of the widely used UV detector has limitations as the compound does not possess UV absorption and has to be derivatized to a UV-absorbing compound [6]. Gas chromatography (GC) method has not been attempted, possibly because of the unstable nature of the endoperoxy group. Thermal stability studies [7,8] have indicated that artemisinin is stable up to 150°C but degrades into number of products when heated at 180–200°C. We have developed a simple and rapid GC method that is based on the indirect detection and determination of artemisinin on the basis of its thermally degraded products, eluted from the column.

EXPERIMENTAL

Materials

Artemisinin was kindly donated by Dr. D. L. Klayman (Walter Reed Army Institute of Research, Washington, DC, U.S.A.). Aretannuin-B was obtained from Dr. R. S. Thakur (Central Institute of Medicinal and Aromatic Plants, Lucknow, India). OV-17 and Gas-Chrom Q (80–100 mesh) were purchased from Applied Science Labs. (State College, PA, U.S.A.).

Gas chromatography

GC was performed on a Shimadzu GC-16 A gas chromatograph fitted with a flame ionization detector. A glass column (2 m × 3.2 mm I.D., silanized) was packed in the laboratory with 3% OV-17 on Gas-Chrom Q (80–100 mesh). Nitrogen was used as the carrier gas at a flow-rate of 40 ml/min. The detector temperature was maintained at 250°C whereas the injector temperature was varied from 200 to 240°C and was maintained at the same level as column temperature. Analyses were carried out isothermally (200, 210, 220, 230 and 240°C) and with temperature programming (100 to 240°C at 4°C/min).

Linear calibration graphs were plotted as the peak areas of the decomposition products *versus* the respective concentrations of the parent compound.

Mass spectral (MS) analyses (electron impact mode) were carried out on a Shimadzu GC-MS QP 1000 mass spectrometer. GC–MS analyses were performed on a 3% OV-17 analytical column with helium as the carrier gas at a flow-rate of 55 ml/min. Both artemisinin and arteannuin-B were analysed using a direct insertion (DI) probe and the GC mode. Artemisinin, in the GC mode, gave a fragmentation pattern for two peaks, A and B. Arteannuin-B gave the same MS pattern in the DI and GC modes. The respective fragmentation patterns were as follows: artemisinin, DI mode, *m/z* 282 (M^+), 250, 232 and 192 and GC mode peak A 282, 269, 224, 211, 195, 192 and 179 and peak B 284, 269, 253, 236, 222 and 204; arteannuin-B, DI and GC modes, *m/z* 248 (M^+), 230, 220, 215, 206 and 190.

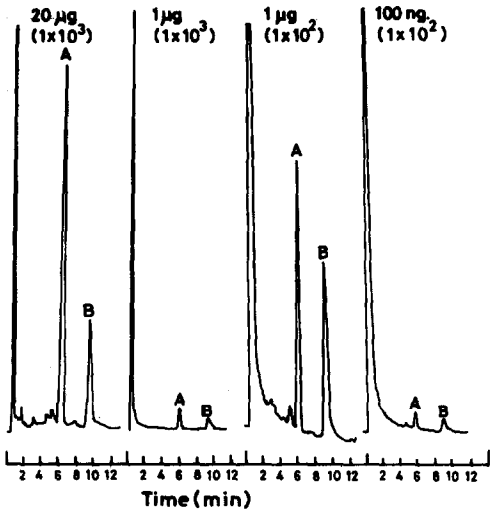

Fig. 1. Isothermal runs on artemisinin (240°C). Different concentrations at two sensitivities (1×10^3 and 1×10^2). A (5.93 min) and B (9.10 min) are the two decomposition products of artemisinin.

RESULTS AND DISCUSSION

The isothermal run with artemisinin (240°C) showed the presence of two peaks, A and B (Fig. 1). The mass fragmentation patterns of A and B, as obtained by GC–MS, did not correspond to that of parent compound. Hence these were artifacts of artemisinin, formed during thermal degradation in the column. With a view to studying the effect of temperature on the thermal stability of the compound, the isothermal runs were repeated at a lower temperature of 200°C and also with temperature programming from 100 to 240°C at 4°C/min. Care was taken to maintain the injector temperature the same as the column temperature. Each time, two peaks were obtained (A and B).

Various concentrations were subjected to GC. A linear relationship was observed between the concentration of artemisinin and the peak areas of its degradation products A and B (Fig. 2). Hence either of the two peaks could be utilized for the detection and determination of artemisinin in unknown samples. Working at the normal sensitivity level (1×10^3), the detection limit was about 1 μg and linearity was observed between 1 and 20 μg of sample. At a higher sensitivity setting (1×10^2), the detection limit was as low as 100 ng.

A problem arises if the plants or the tissue extracts also contain arteannuin-B, because it has the same retention time as A under both isothermal and temperature-programmed conditions. The GC–MS data of arteannuin-B and compound A indicated that the two compounds are different. The presence of arteannuin-B could easily be deduced as it would increase the proportion of A with respect to B. In that event, artemisinin can be determined on the basis of the linear calibration graph for compound B only. Arteannuin-B possesses UV absorption and can easily be detected and determined, at nanogram levels, by HPLC.

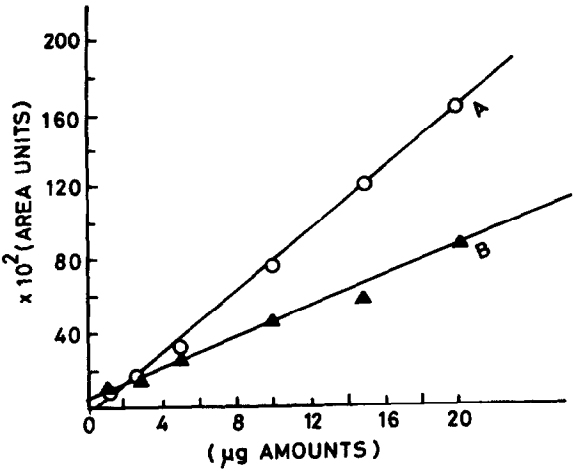

Fig. 2. Linear calibration graph for the two decomposition products (A and B) of artemisinin obtained in an isothermal run (240°C) at 1 × 10^3 attenuation.

This method was applied [9] to the detection and determination of artemisinin in *A. annua* plants and in plantlet cultures developed under different conditions.

REFERENCES

1 X.-D. Luo and C.-C. Shen, *Med. Res. Rev.*, 7 (1987) 29–52.
2 China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials, *J. Trad. Chin. Med.*, 2 (1982) 17 and 45.
3 G. Li, X. Guo, R. Jin, H. Jian and Z. J. Li, *J. Trad. Chin. Med.*, 2 (1982) 125.
4 B. C. Martinez and E. J. Staba, *Adv. Cell Cult.*, 6 (1988) 69–87.
5 N. Acton, D. L. Klayman and I. J. Rollman, *Planta Med.*, 51 (1985) 445–446.
6 S.-S. Zhao and M.-Y. Zeng, *Yaowu Fenxi Za Zhi*, 6 (1986) 3–4.
7 A. J. Lin, D. L. Klayman, J. M. Hock, J. V. Silverston and C. F. George, *J. Org. Chem.*, 50 (1985) 4504–4508.
8 X.-D. Luo, H. J. C. Yeh, A. Brossi, J. L. Flippen-Anderson and R. Gilardi, *Heterocycles*, 23 (1985) 881–887.
9 D. P. Fulzele, A. T. Sipahimalani and M. R. Heble, *Phytother. Res.*, (1990) in press.