Journal of Chromatography, 105 (1975) 95–98

CHROM. 7878

TRIMETHYLSILYLATION OF PENICILLIC ACID AND PATULIN, AND THE STABILITY OF THE PRODUCTS

TOSHIMASA SUZUKI, YOSHINORI FUJIMOTO, YŌJI HOSHINO and AKIO TANAKA
Institute of Public Health, Kamiōkubo, Urawa (Japan)
(Received July 9th, 1974)

SUMMARY

Penicillic acid and patulin have been effectively silylated by N,O-bis(trimethylsilyl)acetamide, alone or with trimethylchlorosilane in benzene. The silylation products are stable, if stored under refrigeration, for at least 15 days.

INTRODUCTION

Penicillic acid and patulin have been successfully analyzed as trimethylsilyl (TMS) ethers by gas–liquid chromatography (GLC) (although with a high detection limit), and various conditions for silylation and GLC have been devised. As the trimethylsilylating reagent, Pohland *et al.*[1] used N,O-bis(trimethylsilyl)acetamide (BSA) for patulin dissolved in ethyl acetate, Suzuki *et al.*[2,3] preferred a mixture of hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS) and pyridine (3:1:5) and Pero *et al.*[4] used BSA, TMCS and pyridine (3:1:9).

The purpose of this work was to study the formation of the TMS derivatives of penicillic acid and patulin by using several reagents and to investigate the stability of the products.

MATERIALS AND METHODS

Reagents

Penicillic acid and patulin were obtained from Wako Pure Chemical Industries (Osaka, Japan), and stock solutions were prepared by dissolving 1 mg of each compound in 10 ml of benzene–acetonitrile (49:1) and diluting with benzene for use.

The combinations of trimethylsilylating reagents examined were (a) BSA–benzene (1:25), (b) BSA–TMCS–benzene (1:1:25), (c) HMDS–TMCS–benzene (1:1:25), (d) N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA)–benzene (1:25), and (e) N-trimethylsilylimidazole (SIM)–benzene (1:25); these reagents were obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan).

Trimethylsilylation

An aliquot of the stock solution was placed in a 10-ml test-tube having a

ground-glass stopper, and the solvent was evaporated under reduced pressure; the dried residue was directly silylated at 20° by adding 1 ml of reagent and allowing the reaction to proceed for 10–15 min. After the reaction, 1 μl of the mixture was injected into the gas chromatograph. The concentrations of penicillic acid and patulin injected were 0.3 and 0.75 ng per μl, respectively.

Gas–liquid chromatography[5]

For GLC, a Shimadzu GC-4BM gas chromatograph with an electron-capture detector (^{63}Ni; 10 mCi) was used. The glass column (2.0 m × 3 mm I.D.) was packed with 10% of DC-200 plus 15% of QF-1 (1:1, v/v) on Gas-Chrom Q (80–100 mesh) and was operated at 175°. The injection part and detector were kept at 190°, and the nitrogen carrier-gas flow-rate was 60 ml per min. The electrometer range was 100 MΩ × 0.16 V.

RESULTS AND DISCUSSION

The chromatograms of the TMS derivatives of penicillic acid and patulin are shown in Fig. 1. The retention time of the penicillic acid derivative was 3.6 min and that of patulin 7.8 min, and the calibration curves were obtained by plotting the peak height against the amount of each[5].

The optimum amount of reagent and the optimum reaction time were investigated by using BSA; the results are shown in Figs. 2 and 3. For 0.3 μg of penicillic acid or 0.5 μg of patulin, at least 20 μl of BSA in 1 ml of benzene was required. The reaction proceeded fairly rapidly and when BSA solution in benzene (40 μl per ml) was added to the solid residue of penicillic acid or patulin, the yield of TMS derivative reached 100% within 10 min.

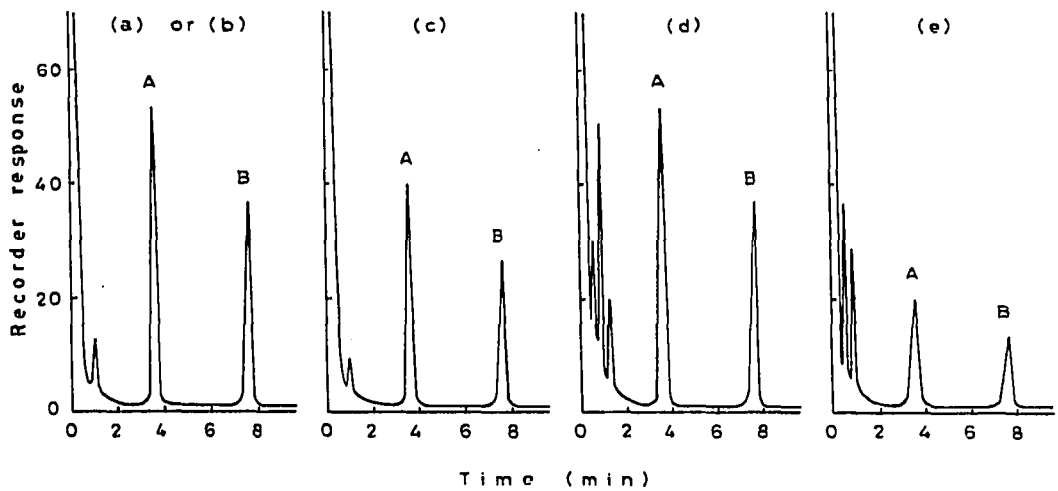

Fig. 1. Gas chromatograms of the TMS derivatives of penicillic acid (A) and patulin (B). The silylating agents added to 0.3 μg of penicillic acid and 0.75 μg of patulin were: (a), BSA; (b), BSA plus TMCS; (c) HMDS plus TMCS; (d), BSTFA; and (e), SIM. The reagents were dissolved in 1 ml of benzene, and the sample size was 1 μl.

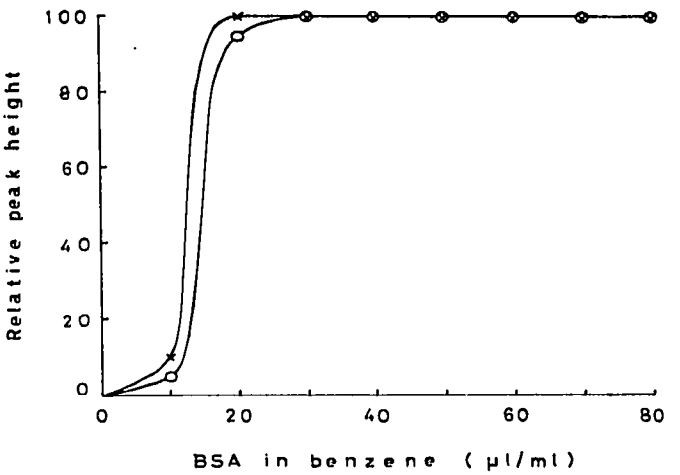

Fig. 2. Effect of amount of BSA on production of the TMS derivatives of penicillic acid and patulin. To 0.3 μg of penicillic acid or 0.5 μg of patulin was added BSA in 1 ml of benzene at 20°, and the products were analyzed by GLC after 1 h. ○—○, Penicillic acid derivative; ×—×, patulin derivative.

In comparing the reactivity of various trimethylsilylating reagents towards penicillic acid and patulin, it was observed that the reaction of reagent (c) was somewhat slower than those with reagents (a) and (b), and that the reaction with reagent (e) was not complete even after 24 h. The chromatograms due shown in Fig. 1 for reagents (d) and (e) include several peaks besides those of the TMS derivatives of penicillic acid and patulin; this suggests the presence of by-products. However, reagents (a) and (b) give good chromatograms and these reagents are suitable for silylating both penicillic acid and patulin.

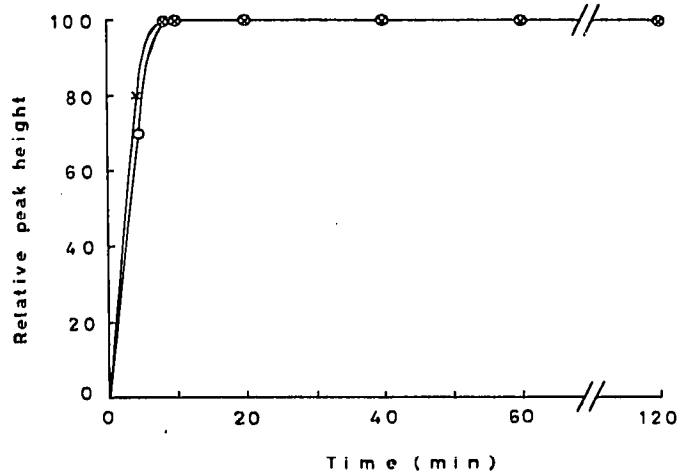

Fig. 3. Time course of the TMS derivative production after addition of BSA to penicillic acid and patulin. To 0.3 μg of penicillic acid or 0.5 μg of patulin, was added BSA solution in benzene (40 μl per ml) at 20°, and the products were analyzed by GLC. ○—○, Penicillic acid derivative; ×—×, patulin derivative.

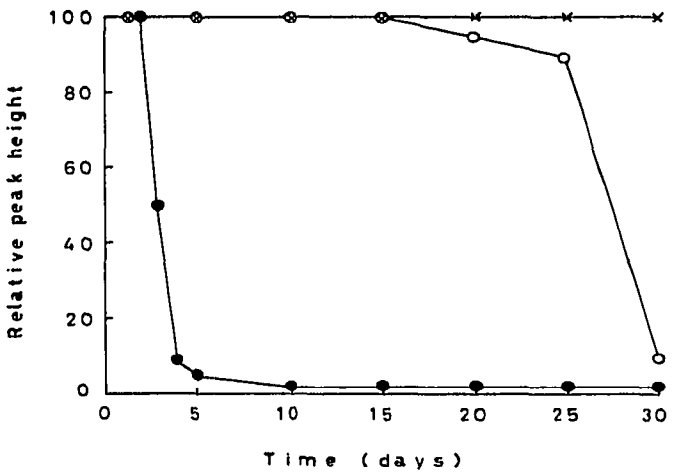

Fig. 4. Effect of storage at 5° or 20° on the TMS derivatives of penicillic acid and patulin. The derivatives were prepared as described for Fig. 3 and were analyzed by GLC. ○—○, Penicillic acid derivative stored at 5°; ●—●, penicillic acid derivative at 20°; ×—×, patulin derivative at 5° or 20°.

The instability of TMS derivatives of penicillic acid and patulin was mentioned by Pohland *et al.*[1] and by Suzuki *et al.*[2], whereas Pero *et al.*[4] and Suzuki *et al.*[3] regarded the derivatives as stable. The derivatives produced from 0.3 μg of penicillic acid and 0.5 μg of patulin by reaction with BSA (40 μl per ml) solution in benzene were therefore stored at 5° or 20°. As shown in Fig. 4, the derivative of patulin was stable even at room temperature for at least one month. The derivative of penicillic acid, however, although stable for about 15 days under refrigeration, was unstable at room temperature, only 2 or 3% of the product remaining after 5 days.

From the results presented above in this paper, it is clear that penicillic acid and patulin were effectively silylated by BSA, either alone or with TMCS, in benzene and that the products could be stored under refrigeration for at least 15 days.

REFERENCES

1 A. E. Pohland, K. Sanders and C. W. Thorpe, *J. Ass. Offic. Anal. Chem.*, 53 (1970) 692.
2 T. Suzuki, M. Takeda and H. Tanabe, *Shokuhin Eiseigaku Zasshi (J. Food Hyg. Soc. Jap.)* 12 (1971) 495.
3 T. Suzuki, Y. Fujimoto, Y. Hoshino and A. Tanaka, *Agr. Biol. Chem.*, 38 (1974) 1259.
4 R. W. Pero, D. Harvan, R. G. Owens and J. P. Snow, *J. Chromatogr.*, 65 (1972) 501.
5 Y. Fujimoto, T. Suzuki and Y. Hoshino, *J. Chromatogr.*, 105 (1975) 99.