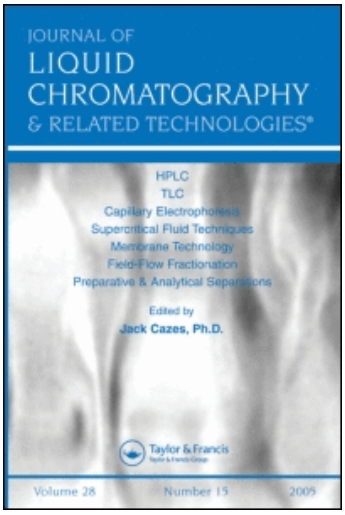

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Reversed Phase Liquid Chromatography of Phenacyl Esters of Some Natural Penicillins

Stanley Lam[a]; Eli Grushka[a]
[a] Department of Chemistry, State University of New York at Buffalo, Buffalo, New York

To cite this Article Lam, Stanley and Grushka, Eli(1978) 'Reversed Phase Liquid Chromatography of Phenacyl Esters of Some Natural Penicillins', Journal of Liquid Chromatography & Related Technologies, 1: 1, 33 — 41
To link to this Article: DOI: 10.1080/01483917808068376
URL: http://dx.doi.org/10.1080/01483917808068376

JOURNAL OF LIQUID CHROMATOGRAPHY, 1(1), 33-41 (1978)

REVERSED PHASE LIQUID CHROMATOGRAPHY OF PHENACYL ESTERS OF SOME NATURAL PENICILLINS

Stanley Lam and Eli Grushka[1]
Department of Chemistry
State University of New York at Buffalo
Buffalo, New York 14214

ABSTRACT

The present work demonstrates a method by which natural, non-UV absorbing penicillins can be derivatized to form phenacyl esters. The reaction is rapid and essentially completed in twenty minutes. A straight line correlation was found between the peak height of a phenacyl ester derivative and the amount of the penicillin, a fact which attests to the applicability of the method for quantitation. The phenacyl esters of the penicillins studied here were separated on a reversed phase column.

INTRODUCTION

The separation and purification of penicillins and cephalosporins from harvesting broths is an important step in the commercial preparation of these antibiotics. Hughes et al. (2) have recently reviewed some of the chromatographic methods used in the analysis of penicillins and cephalosporins. Although high performance liquid chromatography (HPLC) can provide a fast and sensitive method of separation, relatively few workers have applied it to the analysis of penicillins. The work of White et al (3) has clearly demonstrated the superiority of HPLC over spectrophotometric, chemical and microbiological procedures. The usefulness of the method is further exemplified in the analysis of the degradation products of penicillin G(4) and Ampillicin (5). Furthermore, reversed phase HPLC can be used to correlate

biological properties to the partition coefficients of some penicillins and cephalosporins (6).

Thus far, the majority of the penicillins separated by HPLC have a chromophore which can be detected at 254 nm. It is known that several mutants of Penicillin Chrysogenum on synthetic nutrients produce, in addition to the UV absorbing penicillins, several penicillins that give no response to the conventional 254 nm liquid chromatographic UV detectors. These penicillins were previously separated by paper chromatography and identified by the bacterial inhibition method (7). Using an electrical conductivity detector, Martin and Randall (8) have monitored similar penicillins in the eluent of a liquid chromatographic column. Recently, a gas-liquid chromatographic method was reported for the determination of the methyl esters of natural penicillins with aliphatic side-chains together with penicillin G and V (9).

Liquid chromatography can be more attractive for the analysis of penicillins since it allows easy trapping procedures for those antibiotics which have to be characterized further. Therefore, it would be beneficial to develop a derivatization scheme which would permit the use of UV detectors to analyze natural alkyl-penicillins.

Phenacyl derivatives are known for their excellent chromatographic properties and detectabilities. They have been previously applied to fatty acids (viz. 10,11) and prostaglandins (12). Taking advantage of the carboxylate moiety present in penicillins, the phenacyl derivatives can be prepared easily with slight modifications of the procedures reported before (10, 12). This paper reports the preparation of phenacyl penicillin derivatives and their separation on a reversed phase column.

EXPERIMENTAL

Chemicals and reagents: Penicillin K (n-heptyl-penicillin), penicillin F (n-pent-2-enyl-penicillin), and the potassium salts

of dihydro- penicillin F(n-pentyl-penicillin) and penicillin V (phenoxymethyl penicillin) were provided by Bristol Laboratories (Syracuse, N.Y.). Triethylamine and heptaphenone were obtained from Eastman-Kodak (Rochester, N.Y.). α,p-dibromoacetophenone and 18-Crown-6 were purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin). All organic solvents were obtained from Fisher Scientific (Pittsburg, Penn.). All chemicals were used as received with the exception of acetonitrile which was dried with molecular sieve when used as a derivatization solvent. Water used for the mobile phase was distilled and passed through an ion exchange column (Barnstead Sybron, Boston, Mass.).

Instrumentation. The liquid chromatograph was assembled in our laboratory. The gradient system used was a simplification of the one described by Scott (13). Acetonitrile was introduced at a rate of 1.5 ml/min. to a mixing vessel (50 ml beaker with a magnetic stirrer) through a 50 ml buret. The initial condition in the mixing vessel was 20 ml of 5% CH_3CN in water. The mixed solvent was delivered to the column using a model 396 Milton Roy mini pump (LDC, Riveria Beach, Fla.) at a flow rate of 1.3 ml/min.

The samples were introduced via a Rheodyne injection valve model 70-10 purchased from Altex Scientific Co. (Berkeley, Ca.). The size of the injection was 10 µl. The UV detection at 254 nm was carried out with a Tracor 100 detector (Tracor Inc., Austin, Texas) having a cell volume of 8 µl. The reversed phase packing was prepared by reacting trimethoxyoctadecylsilane with Partisil 10 (Whatman, Clifton, N. J.). A 250 nm x 4.2 mm I.D. column was packed by the conventional slurry technique.

Derivatization Procedures. The following stock solutions were prepared: 0.21 gm of α,p-dibromoacetophenone in 100 ml of acetonitrile, and 0.07 gm of 18-crown-6 in 100 ml of acetonitrile. Two different procedures were used to derivatize the penicillins depending on whether the penicillin was available as the free acid or as the potassium salt.

Alkylation of Penicillin as the Potassium Salt. One ml of the crown ether stock solution was added to approximately 6 mg of the penicillin in 10 ml of acetonitrile. The mixture was heated to 80°C, at which point 5 ml of α,p-dibromoacetophenone stock solution were added. The reaction was allowed to continue for 30 minutes. The cooled reaction mixture could then be chromatographed or refrigerated for later analysis.

Alkylation of Penicillin as the Free Acid. The same proportions of penicillin to α,p-dibromoacetophenone were used. The same procedure as the above method was followed with the exception that 3 drops of triethylamine were added in place of crown ether to catalyze the reaction.

RESULTS AND DISCUSSION

Initial attempts to neutralize the free acid groups of the penicillins with methanolic-KOH to produce the potassium salt for subsequent crown ether catalyzed alkylation (10) proved to be a failure. When the product of such an alkylation reaction was chromatographed, several peaks result, and the derivatized penicillin could not be identified easily. The extra peaks observed are attributed to degradation products since penicillins are known to break down rapidly in an hydroxide solution (14). When the penicillin is available as its potassium salt, crown ether catalyzed alkylation can be carried out. Otherwise, a second route utilizing a small amount of triethylamine is preferred. This alternative method produces derivatives with no observed decomposition products.

The derivatization reaction, to be of practical value, should be fast and its yield should be proportional to the amount of the starting penicillins. To study the reaction rate and the derivative formation, controlled quantitites of heptaphenone were used as internal standard. The heptaphenone was introduced to the mixture prior to the addition of alkylating agent. The amount of derivatized product is, therefore, measured relative to

the internal standard. When the reaction rate was studied, samples were taken directly from the reaction flask at certain times, and either chromatographed at once or refrigerated for later analysis after cold methanol was added to quench the reaction.

The extent of the reaction as a function of time for both alkylation methods is shown in Figure 1. The peak height ratio is defined as the height of the penicillin peak to that of the internal standard, namely heptaphenone. The increase in the peak height of the penicillin as compared to the constant internal standard gives a measure of the yield of the reaction. The peak height ratio vs. time plots show that the reaction is essentially completed in about 20 minutes. It seems that the triethylamine route progresses initially at a faster rate.

Penicillin V, which absorbs UV at 254 nm, was used to monitor the completeness of the reaction. Figure 2 shows chromatograms of the reaction mixture at 50 sec. and at 1800 sec. after the start of the reaction. Figure 2a shows that after 50 sec. penicillin

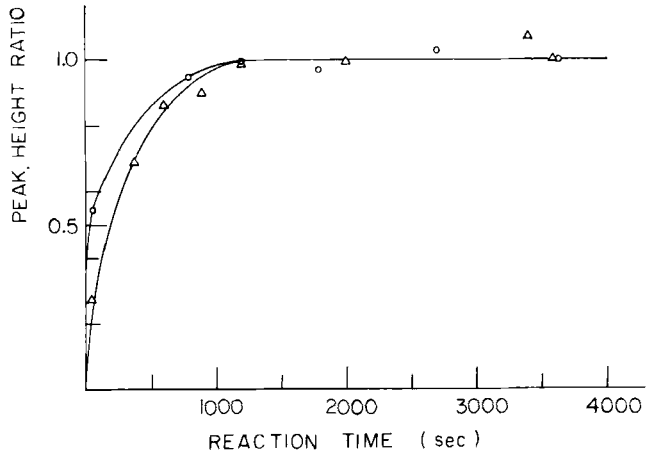

FIGURE 1

Rate of reaction of penicillin with α,p-dibromoacetophenone. O - Penicillin F catalized with triethylamine. Δ-Penicillin V catalyzed with 18-crown-6.

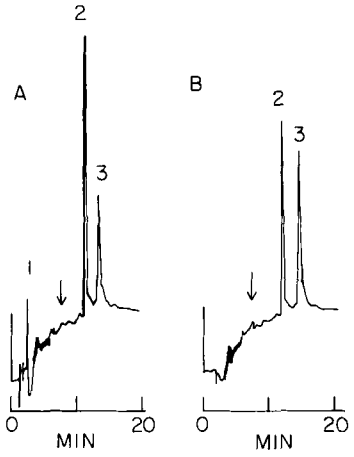

FIGURE 2

Separation of penicillin V phenacyl ester from its starting material. A = 50 sec. after the start of the reaction. B = 1800 sec. after the start of the reaction. At the point indicated by an arrow, the attenuation was changed from 0.16 AUFS to 0.32 AUFS. Mobile phase 5% acetonitrile in water increasing to 76% acetonitrile (see text). (1) - Penicillin V, (2) - α,p-dibromo-acetophenone, (3) Phenacyl ester of penicillin V.

V is present in the mixture together with the alkylating reagent and the phenacyl derivative. After 30 minutes no penicillin V could be detected at the attenuation used. The amount of the derivative is larger in Figure 2b as compared to 2a while the peak corresponding to the unreacted dibromoacetophenone decreased. The mobile phased used in Figure 2 was an acetonitrile gradient. The gradient was formed before the pump and the composition of the mobile phase was calculated according to the method described by Scott (13). It is interesting to note the large differences in the retention times of the free and derivatized penicillin.

Figure 3 shows a plot of the height of the phenacyl ester of penicillin V peak relative to that of heptaphenone for four initial amounts of penicillin V. The linear plot indicates that the present method of derivatization can be used for quantitative purposes.

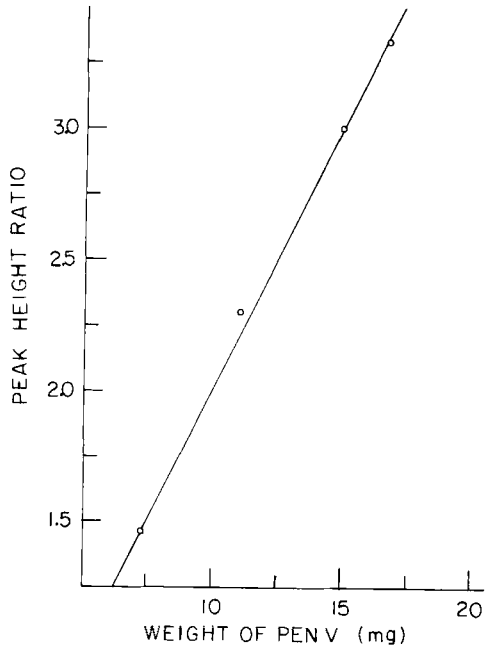

FIGURE 3

Peak height ratio versus mg of penicillin V used in the derivatization reaction.

The separation of the phenacyl esters of three alkylpenicillins is shown in Figure 4. No attempts were made to optimize the resolution or to decrease the retention times. No studies were made to investigate the limits of detection; however, our previous work with carboxylic acids (10, 15) would seem to indicate that 10^{-5} gm of the penicillins per ml of solvent could easily be detected.

In summary, it seems that penicillins can be derivatized to form the phenacyl esters. The reaction is fast and seems to go to completion. As a consequence, alkyl penicillins, once derivitized, can be chromatographed, and quantitated with the commonly used 254 nm UV detector.

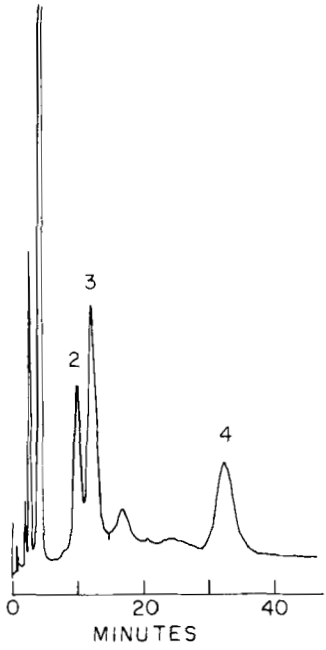

FIGURE 4

Separation of phenacyl esters of some aliphatic penicillins. UV detector at 254 nm, 0.32 AUFS. Mobile phase 40% water in MeOH. Flow rate 2.2 ml/min. (1) - α,p-dibromoacetophenone, (2) - penicillin F, (3) - dihydropenicillin F, (4) - penicillin K.

ACKNOWLEDGEMENT

The authors would like to thank Bristol Laboratories for the gift of the penicillins.

REFERENCES

(1) To whom all correspondence should be addressed.

(2) Hughes, D. W., Vilim, A. and Wilson, W. L., Canad. J. Pharm. Sci., 11, 97, (1976).

(3) White, E. R., Carrol, M. A., Zarembo, J.E., and Bender, A. D., J. Antibiotics, 28, 205 (1975).

(4) Blaha, J. M., Knevel, A.M., and Hem. S. L., J. Pharm. Sci., 64, 1384 (1975).

(5) Tsuji, K., and Robertson, J. H., J. Pharm. Sci., 64, 1542, (1975).

(6) Yamana, T., Tsuji, A., Miyamoto, E., and Kubo, A., J. Pharm. Sci., 66, 747, (1977).

(7) Karnovsky, M.L., and Johnson, M. J., Anal. Chem. 21, 1125 (1949).

(8) Martin, A.J.P., and Randall, S.S., Biochem. J. 49, 293 (1951).

(9) Meesschaert, B., Adriaens, P., and Eyssen, H., J. Chromatogr. 136, 1962 (1977).

(10) Durst, H.D., Milano, M.J., Kikta, E. J., Jr., Connelly, S. A., and Grushka, E., Anal. Chem. 47, 19797 (1975).

(11) Borch, R. F., Anal. Chem., 47, 2437 (1975).

(12) Morozowich, W., and Douglas, S. L., Prostaglandins, 10, 19, (1975).

(13) Scott, R.P.W., J. Chromatogr. Sci., 9, 385 (1971).

(14) Yaman, K., Tsuji, A., Kiya, E., and Miyamoto, E., J. Pharm. Sci., 66, 861 (1977).

(15) Grushka, E., Durst, H. D., Kikta, E. J. Jr., J. Chromatogr. 112, 673 (1975).