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APPLICATION OF PHENACYL DERIVATIVES FOR THE DETERMINATION OF FATTY ACIDS BY HPLC

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INTRODUCTION

Chemical derivatization, a technique by which the physical and chemical characteristic of the analyte is changed to give better chromatographic properties, is important in the development of gas chromatographic (GC) and liquid chromatographic (LC) methodologies. Many recent reviews on GC (1-8) and LC (6-15) derivatization approaches have been reported.

The common detectors in LC are the UV-visible spectrophotometer, differential reflectometer, fluorometer and electrochemical detector. These devices, with the exception of the reflectometer, are selective detectors which respond only to specific solute properties. Some compounds would pass these LC detectors unnoticed, and give unsatisfactory analysis. Amino acids, biogenic amines, fatty acids, sugars and lipids for instance, are transparent to the fixed wavelength 254 and 280 nm UV detectors and generally possess no fluorescence. Although most of these compounds can be detected between

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190-210 nm, the sensitivity is inadequate. Further, in this part of the UV spectrum, the mobile phase requirement is stringent. Only a few of the buffer salts and organic solvents with low UV cutoffs may be added to the eluent without introducing an absorbing background. Alternatively, these natural products can be analyzed by forming a derivative with a fluorophore or chromophore for more sensitive detection. Chemical derivatization also eliminates acid or base dissociation of the solute in the mobile phase, and improves the chromatographic efficiency, separation and peak symmetry.

Fatty acids are important in various aspects of biochemical studies. Free fatty acids have been determined by LC with UV monitored at 210 nm (16, 17), but they are most frequently assayed as the derivative of a chromophore or fluorophore. By far, the most popular UV chromophores are the phenacyl halides and the fluorophore is 4-bromomethyl-7-methoxycoumarin. In this article, we shall review the preparation of fatty acid phenacyl esters and their applications in HPLC.

PREPARATION OF PHENACYL ESTERS

Shriner, et al.(18) described the traditional procedure for preparing the phenacyl ester by reacting the salt of an acid with the corresponding halide in an alcoholic solution. The phenacyl ester has also been obtained by reacting the sodium salt of a fatty acid with the appropriate α-bromoacetophenone in DMF (19). However, these methods suffer from several drawbacks: 1) phenacyl halides hydrolyze to phenacyl alcohols in the presence of an alkali base during the reaction; 2) the reaction is slow and the yield not quantitative; 3) abnormal results occur when the reaction is conducted in the presence of excess sodium chloride (20). For these reasons, the classical procedures for preparing phenacyl esters have not been used for the LC quantitation of fatty

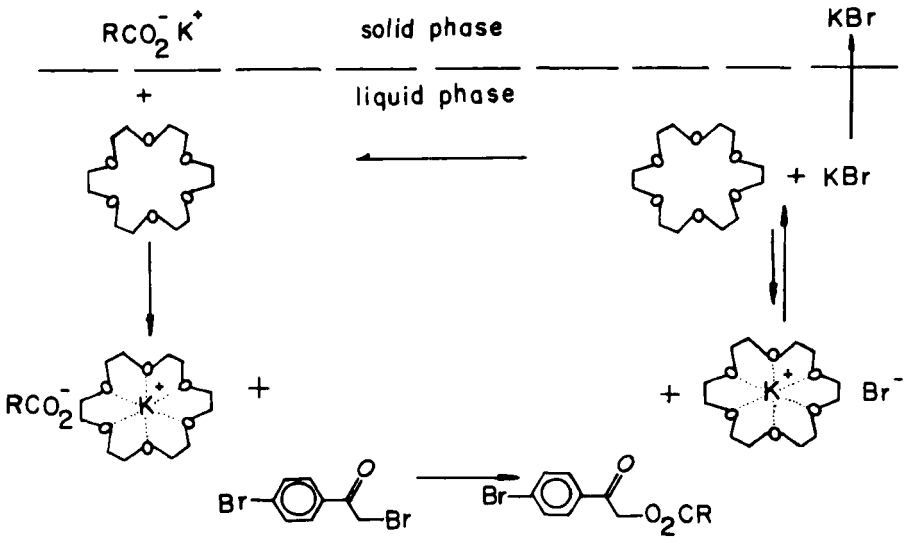
acids. Instead two improved procedures, one using a crown ether and the other a tertiary amine catalyst, have been developed to circumvent the above shortcommings.

Crown Ether Phase Transfer Catalysis

Durst, et al. (21), in measuring fatty acids by HPLC, introduced crown ether as the phase transfer catalyst for the derivatization of carboxylates with α,p-dibromoacetophenone (Scheme I). The crown ether, forming a complex with the potassium ion, extracts the carboxylic salt from its solid phase into the organic solvent (22). The metal ion is enclosed in the solvation envelope of the crown ether. The carboxylate anion, in the weakly solvated reaction environment, is naked and highly reactive. It alkylates the phenacyl bromide to form the corresponding ester in good yield (23, 24).

The crown ether in Scheme I is 18-crown-6. Crown ethers such as the dicyclohexyl-18-crown-6 or the dibenzo-18-crown-6

SCHEME I



can also be used. In practical consideration, the crown ether should be soluble in the reaction solvent and should not, as the result of co-elution with the phenacyl ester of interest, cause absorption interference. It should be emphasized that a stoichiometric concentration of the crown ether to the alkylating agent is not required in the phase transfer reaction (23, 24). Alkylating agent/crown ether in molar ratios of 10:1 to 20:1 can be used.

The sodium salt of a fatty acid, notwithstanding a weaker complex with the crown ether catalyst, also reacts smoothly with the phenacyl halides to give derivatives in good yields (23, 25, 26). Sodium valproate was derivatized in 20 minutes at 70 °C (26). Potassium methoxide has also been used as a base to label steroidal acids (27).

The solvents most often used for the derivatization reaction are benzene, acetonitrile and acetone, although other aprotic solvents may be interchangeable (21). The ideal solvent is one that would solubilize the reactants and products, and is miscible with the mobile phase in the subsequent LC analysis.

The reaction kinetics of the potassium Penicillin V with α,p-dibromoacetophenone catalyzed by 18-crown-6 in acetonitrile at 82° C is shown (Figure 1). The plot of the peak height ratio of Penicillin V/ heptaphenone (internal standard) versus time, shows the completion of reaction in 20 minutes (28).

The phase transfer catalysis is quantitative. Isolated yields of phenacyl esters between 90-100% have been reported (23). On the derivatization of Penicillin V with phenacyl bromide, the completeness of the reaction was also checked by a chromatographic method (28). Both the reactant and product, which absorb UV, were monitored at 254 nm. The chromatogram from the 50 second reaction indicates that Penicillin V, the alkylating agent and the phenacyl ester were present (Figure 2A). The chromatogram from the 1800 second reaction shows that Penicilin V disappeared while the phenacyl ester increased and the unreacted α,p-dibromo- acetophenone diminished at the completion of the reaction (Figure 2B).

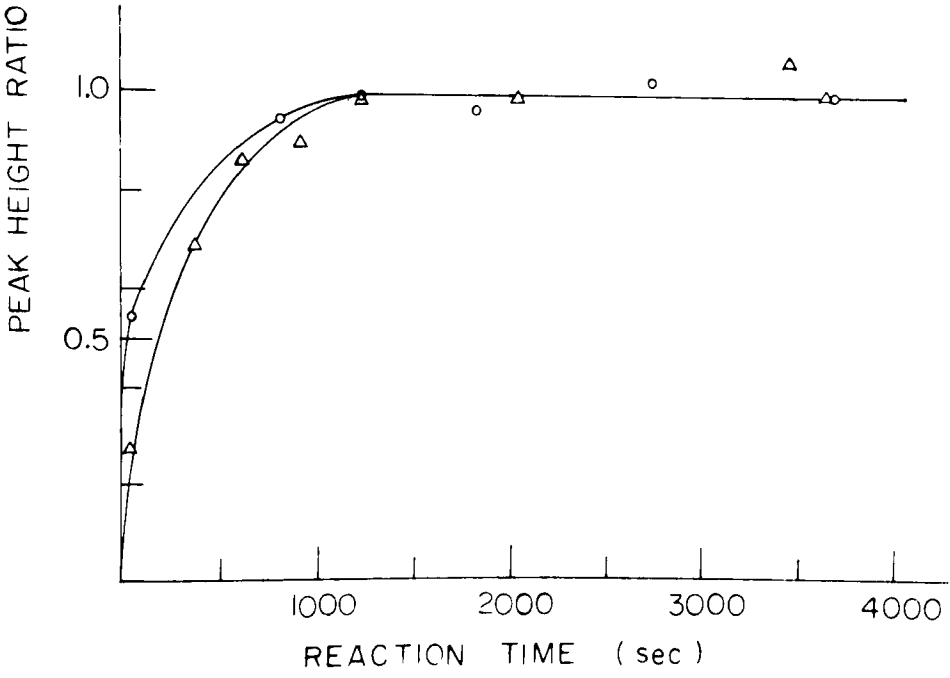


FIGURE 1

Rate of reaction of Penicillin F with α,p-dibromoacetophenone.
Circle - catalyzed by triethylamine.
Triangle - catalyzed by 18-crown-6
After Lam and Grushka (28).

Phase Transfer Derivatization Procedures

The following procedures are used for the derivatization of free fatty acids and their metal salts:

Method A - Alkylation of a Monocarboxylic acid after KOH neutralization

A sample of the organic acid dissolved in methanol is neutralized to the phenolphthalein end point by a 10% KOH-methanoic solution in a reaction flask. The potassium salt of the carboxylic acid is obtained after the evaporation of

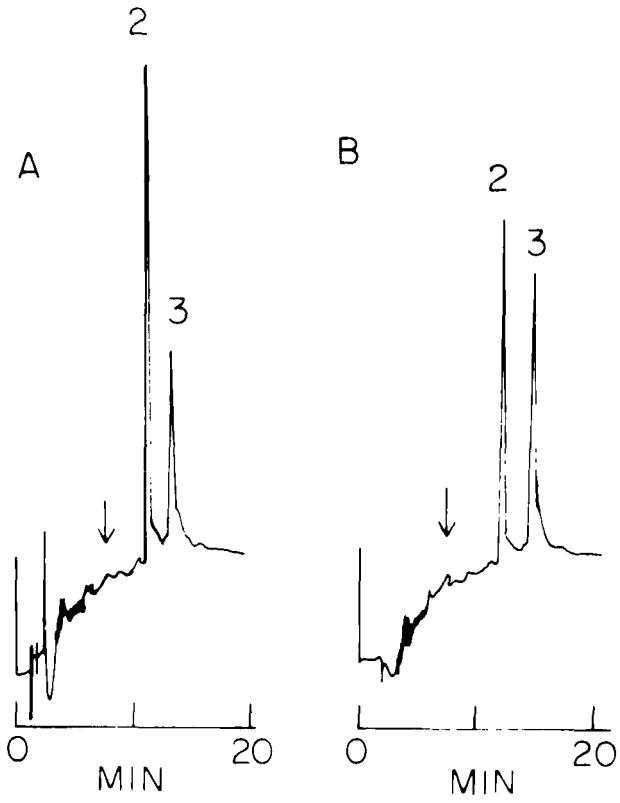


FIGURE 2
Monitoring the formation of Penicillin V phenacyl ester at two different time during the course of reaction. 2A is 50 seconds after the start of the reaction and 2B is 1800 seconds. At the point indicated by the arrow, the attenuation was changed from 0.16 AUFS to 0.32 AUFS. Mobile phase: 5% acetonitrile in water increasing to 76% in 20 minutes. (1) Penicillin V, (2) α,p-dibromoacetophenone, (3) Penicillin V phenacyl ester. After Lam and Grushka (28).

methanol. The reaction is initiated by the addition of a 20% excess derivatizing agent consisting of phenacyl bromide/crown ether 10:1 molar ratio in acetonitrile. The final volume of the reaction mixture can be brought up to 5 or 10 ml with the appropriate solvent. The actual reaction volume is not critical

as long as it would sustain a smooth reflux at 80° C. The reaction time is 15 minutes. The 20% excess derivatizing agent is adequate in giving a quantitative yield without producing an overshadowing reagent peak in the chromatogram.

Method B - Alkylation of a Fatty acid in the Presence of Solid K_2CO_3

This procedure derivatizes a free acid without the prior preparation of a salt. Potassium carbonate in concentrations 3-5 times that of the free acid is added to a reaction flask or a Reacti-Vial. An excess of the derivatizing agent is added. The reflux time at 80°C is extended to 25 minutes. Sodium bicarbonate (26) and potassium methoxide (27) have also been used in place of potassium carbonate to give satisfactory results.

Method C - Alkylation of a Fatty acid salt

A fatty acid salt can be derivatized directly by the addition of the alkylating agent and the crown ether catalyst in an appropriate solvent. The reaction time is 25 minutes as in Method B.

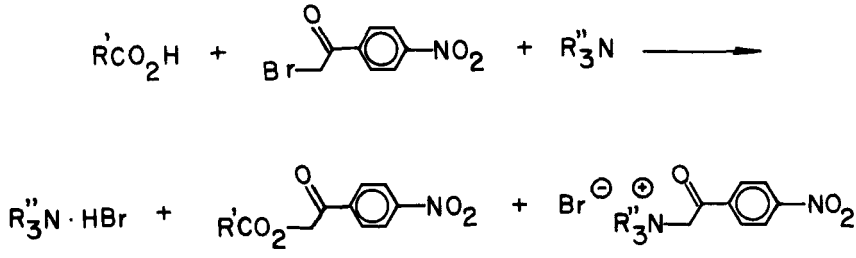
Method D - Alkylation of a Dicarboxylic acid

A dicarboxylic acid can be derivatized with the general method described for a monocarboxylic acid by increasing the alkylating agent and the crown ether concentrations by a factor of two to compensate for the additional carboxyl functional group. Likewise, the reaction time should be doubled. Therefore, the reaction time is 30 minutes by KOH neutralization and 1 hour by the addition of solid potassium carbonate.

Tertiary amine catalyst

The second procedure for the preparation of phenacyl esters uses tertiary amines as catalysts (Scheme II). The tertiary amine R''_3N can be N,N-diisopropylethylamine (29) or

SCHEME II



triethylamine (28). The tertiary amine $R"_3N$, besides acting as an HBr scavenger, also forms a quaternary ammonium species with the phenacyl bromide. N,N-diisopropylamine, which forms the quaternary ammonium species (Scheme II) in a lower rate than triethylamine, is preferred, because less phenacyl halide would be lost over to the side product (Figure 3). Nevertheless, several fold molar excess of the alkylating agent is generally used to allow for the reagent loss.

A standard procedure for the preparation of phenacyl esters using tertiary amines as catalysts has been described (30). Organic acid/derivatizing agent/tertirary amine in molar ratios of 1:4:2 in dimethylformamide are allowed to react at 60°C for one hour. The same reagent ratios can be used for prostaglandins. The yield is 95% for one hour reaction at 25°C (31). Our experiment with triethylamine for the preparation of penicillin phenacyl esters showed that the reaction required 20 minutes (28).

The crown ether phase transfer reaction is fast and quantitative. The procedure is simple and easy. Micro samples that are difficult to neutralize and manipulate can be alkylated in the presence of potassium carbonate. Because of its simplicity, the preparation of phenacyl esters by the crown

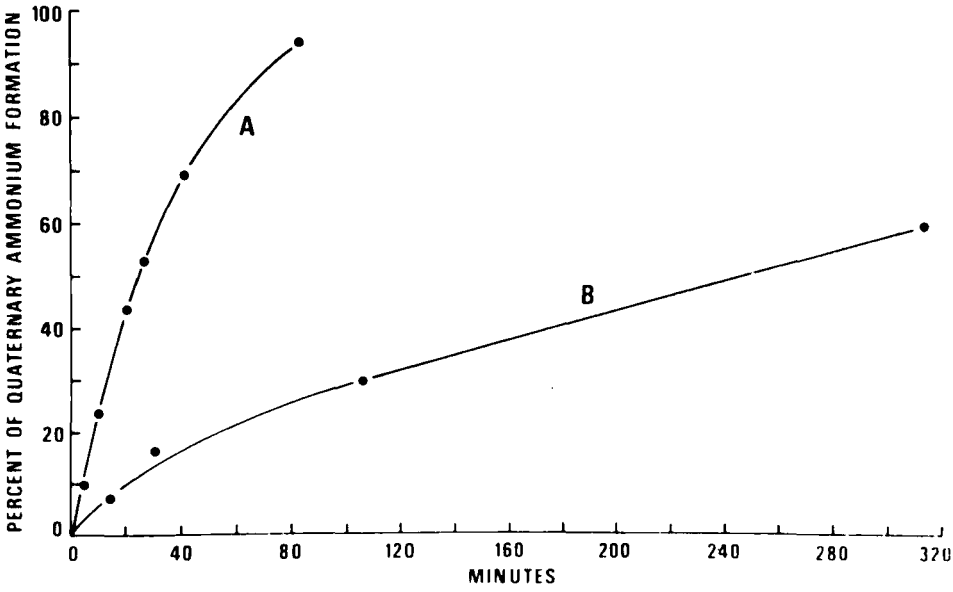


FIGURE 3
Comparison of the rate of reaction of triethylamine and N,N-diisopropylethylamine with p-nitrophenacyl bromide in 1 ml acetonitrile at room temperature. The percent of quaternary ammonium formation was monitored by quenching aliquots with acid followed by back titration with base.
After Morozowich and Douglas (29).

ether potassium carbonate procedure is most often recommended.

The tertiary amines are excellent alternatives to the crown ethers for the catalatic alkylation of fatty acids especially when the samples are heat or alkaline base labile. The major drawback of the tertiary amine catalyst is the requirement of a large excess of the alkylating agent which, upon injected on the column, give a large interfering peak. An additonal sample clean up step using silica gel column chromatography (29) may help to remove the excess reagent and alleviate the interference.

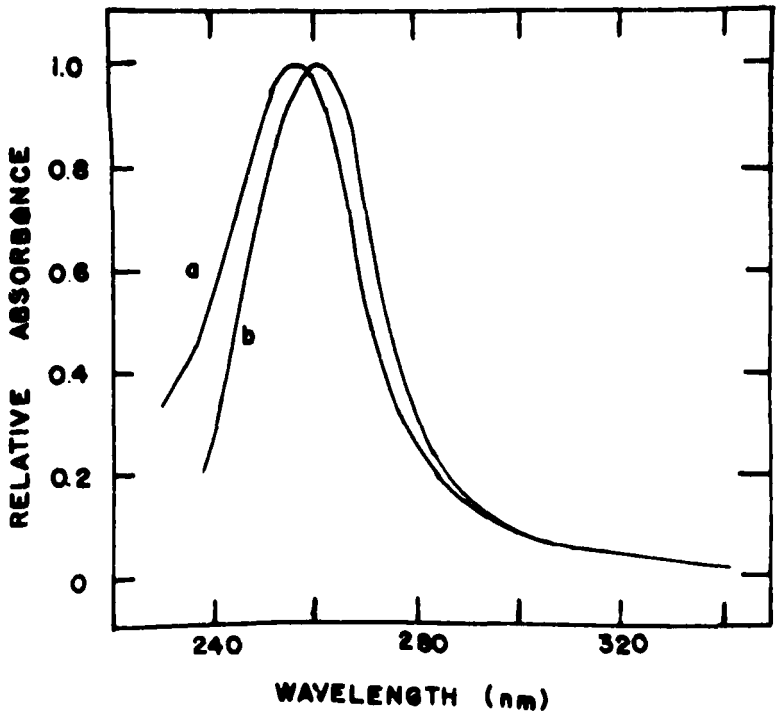


FIGURE 4
UV spectra of propionic acid derivative, a) in methanol and b) in chloroform.
After Durst, Milano, Kikta, Connolly and Grushka (21).

PHENACYL ESTER APPLICATIONS

Mono Carboxylic Acids

The UV spectra of propionic p-bromophenacyl ester in, A) methanol, and B) chloroform (21), are shown (Figure 4). The peak maximum appears at 257 nm in methanol and at 261 nm in chloroform. The molar absorptivity is 18,700 $\pm$ 126 l $mol^{-1}cm^{-1}$ due to the shift of absorption peak maximum in chloroform. Thus, phenacyl esters are very useful derivatives for the LC determination of fatty acids, and give excellent responses on the fixed wavelength 254 nm detector.

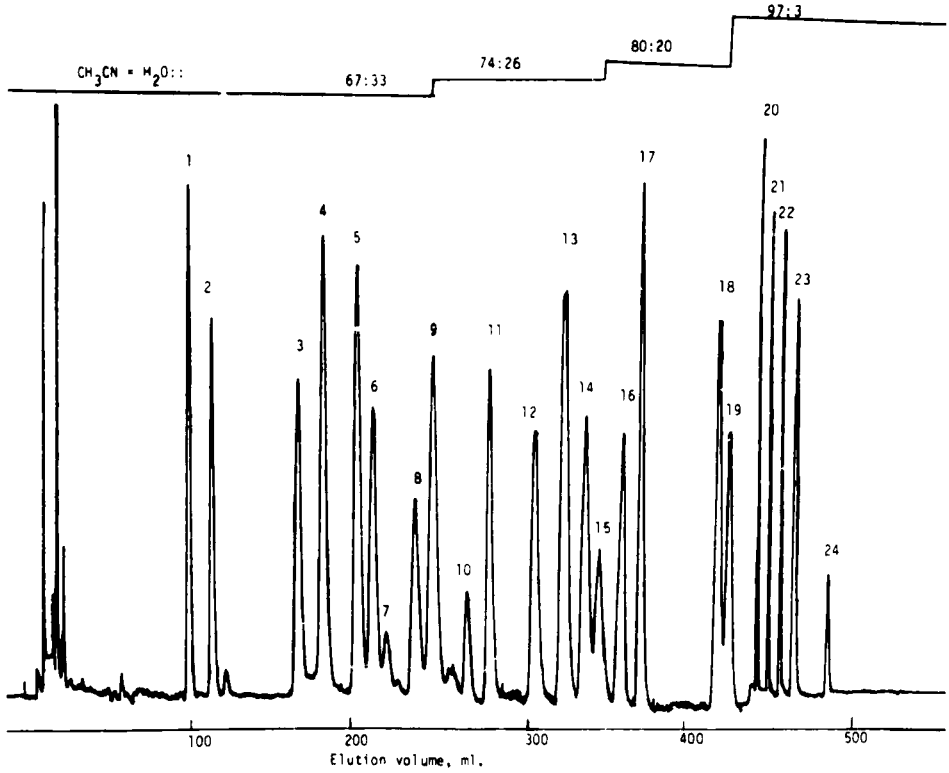


FIGURE 5

Separation of fatty acid phenacyl esters. (1) lauric (12:0); (2) myristoleic (14:1); (3) α- and γ-linolenic (18:3); (4) myristic (14:0); (5) palmitoleic (16:1); (6) arachidonic (20:4); (7) trans-palmitoleic (trans 16:1); (8) linoleic (18:2); (9) pentadecanoic (15:0); (10) linolelaidic (trans 18:2); (11) eicosatrienoic (20:3); (12) palmitic (16:0); (13) oleic (18:1 Δ^9) and vaccenic (18:1, Δ^{11}); (14) petroselinic (18:1, Δ^6); (15) elaidic (trans 18:1); (16) eicosadienoic (20:2, $\Delta^{11,14}$); (17) heptadecanoic (17:0); (18) stearic (18:0); (19) eicosaenoic (20:1, Δ^{11}); (20) nonadecanoic (19:0); (21) arachidic (20:0) and erucic (22:1); (22) heneicosanoic (21:0); (23) behenic (22:0) and nervonic (24:1); (24) lignoceric (24:0). Column 90 x 0.64 cm μ-Bondapak C-18; eluent acetonitrile-water; flow rate 2.0 ml/min. After Borch (32).

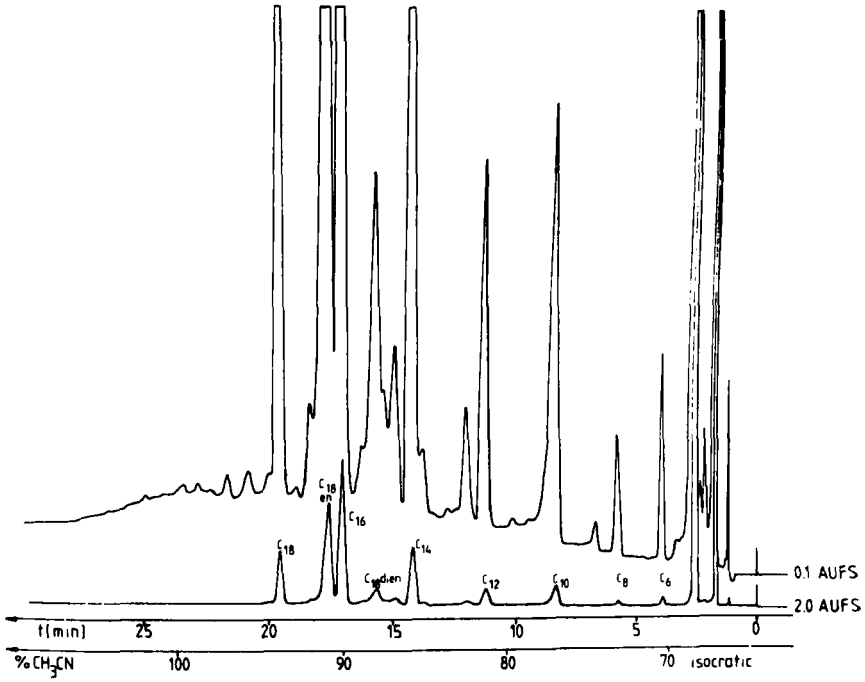


FIGURE 6
Gradient elution of the fatty acid phenacyl esters from butter. Column C-8 RP on Si 100, 30 x 0.42 cm. Gradient volume, 40 ml; flow rate, 2 ml/min. Starting eluent: 70% acetonitrile in water, linear gradient to 100 % acetonitrile in 20 minutes. After Engalhardt and Elgass (34).

The isocratic separation of short chain and long chain fatty acids by adsorption and reversed phase chromatography has been described by Durst et al.(21). Using a step gradient consisting of water and acetonitrile and a μ-Bondapack C_{18} column, 24 long chain fatty acids were separated (Figure 5) (32). The four hour analysis separated fatty acids by the chain length, the degree of unsaturation, and the manner of substitution on the double bonds. The reversed phase chromatographic retention is in favor of long chain over short chain fatty acids, and mono-unsaturated over poly-unsaturated

fatty acids with the cis over the trans isomer. Further, an unsaturated fatty acid with the double bonds close to the carboxyl group is retarded more on the column than a positional isomer with the double bonds distant from the carboxyl group.

Sophisticated solvent gradient is the standard approach for fatty acid profiling in biological samples (33,34). Engelhardt and Elgass have described the optimal conditions for the gradient separation of phenacyl esters (34). The best result was achieved with a 70-100% linear gradient of acetonitrile. The retention of fatty acids is characteristic of the gradient volume. Engelhart and Elgass recommended a gradient volume of 20-60 ml for a 25 cm column for a simple separation, and a gradient volume of 100-200 ml for a complex multi-component analysis of fatty acids. A pattern showing the fatty acid distribution in butter obtained by a water acetonitrile gradient is shown (Figure 6). All of the major components could be quantitated except butyric acid which co-eluted with the reagent peak.

In establishing chemotaxonomy of microorganisms, many studies have been focused on the fatty acid composition by GLC. However, the limitation of GLC is that it cannot be applied to fatty acid methyl esters much above C_{32}. Recently, LC has been used for the profiling of microbial fatty acids as their phenacyl esters. A chromatogram of the long chain fatty acids in Streptococcus mutans 27175 is shown (Figure 7) (35). The methoxyphenacyl esters provided fatty acid quantitation and indentification of the microbes. Bussell and co-workers have also separated hydroxyl fatty acids in oral bacteria (36). The complex chromatogram of β-hydroxyl long chain fatty acid is shown (Figure 8). The fatty acid composition of V. parahaemolyticus, a gram-negative estuarine micro-organism associated with seafood-born enteritis in man, has been obtained (37). The major fatty acid contents found by the LC procedure are in general agreement with GC methods. The derivatization technique is a valuable tool in the further identification and differentiation of microorganisms which are closely related to

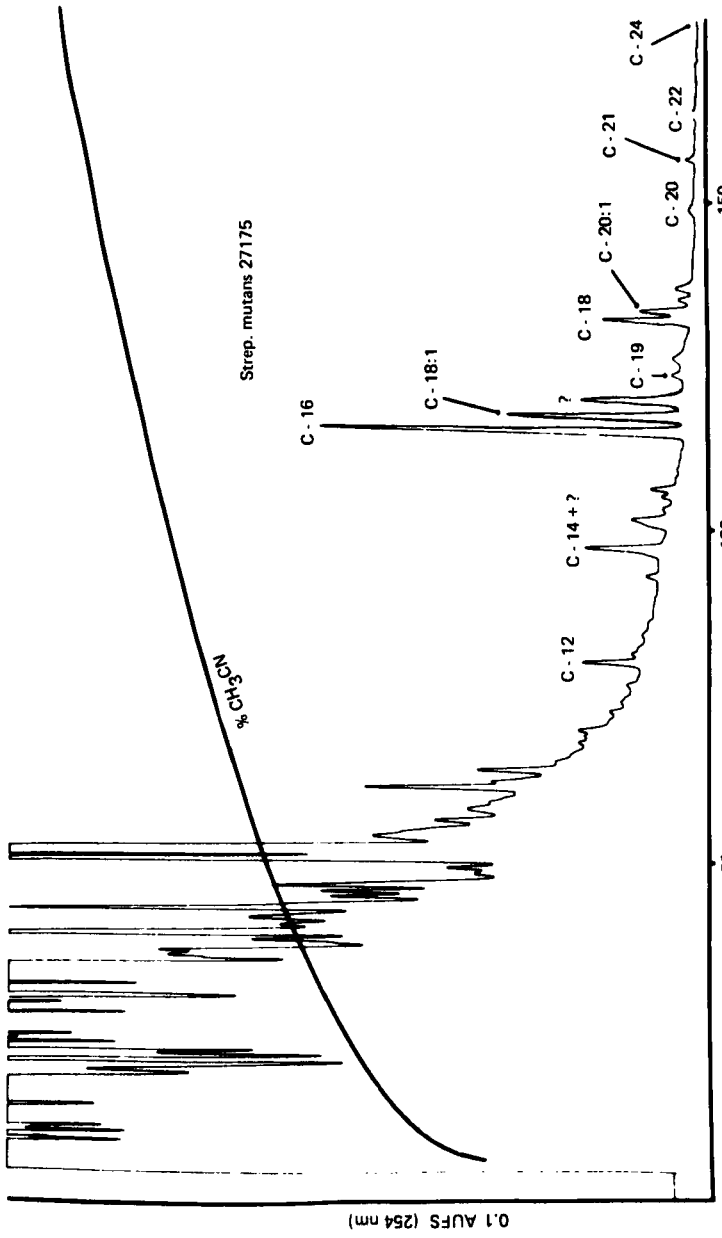


FIGURE 7: Chromatogram of the fatty acids in Streptococcus mutans ATCC 27175, Column: two μ-Bondapak C-18; Mobile phase: acetonitrile water gradient at 1 ml/min. After Miller, Bussell and Ricketts (35).

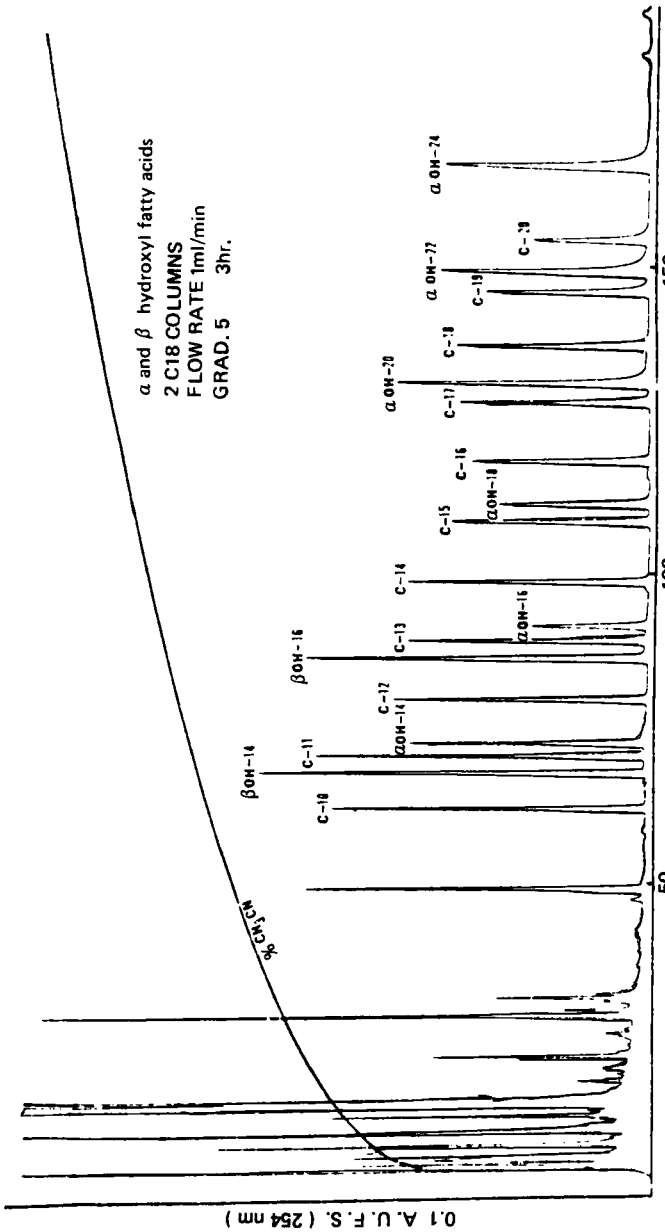


FIGURE 8

HPLC of TFAA hydroxyl fatty acids. Column: two 30 x 0.39 cm μ-Bondapak fatty acid analysis column; eluent acetonitrile: water, gradient curve 5, 6 hours; flow rate 1.0 ml/min; column temperature 38° C. TFAA hydroxyl fatty acids are indicated by asterisk and an underline.
After Bussell and Miller (36).

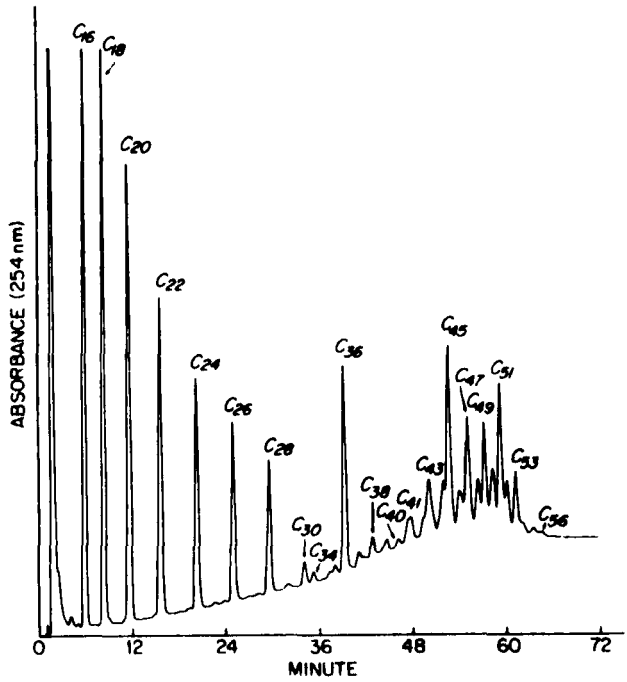


FIGURE 9
HPLC fractionation of a reconstituted mixture of p-bromophenacyl esters of saturated C_{16} to C_{56} fatty acids. A C_{18} bonded silica cartridge was used. The mobile phase was a linear gradient of 0 to 70% p-dioxane in acetonitrile in 60 min at a flow rate of 3 ml/min. After Qureshi, Sathyamoorthy and Takayama (40).

each other and cause taxonomic confusion.

HPLC is superior to GC in analyzing long chain fatty acids. Many long chain fatty acids that had not been previously purified by GLC due to high molecular weight and low volitility were recently found by LC techniques. Takayama et al. (38, 39) have separated the p-bromophenacyl esters of saturated C_{35}-C_{56} fatty acids from Mycobacterium tuberculosis H37Ra (Figure 9). Subsequently, the detailed structures of these compounds were characterized by NMR and mass spectrometric

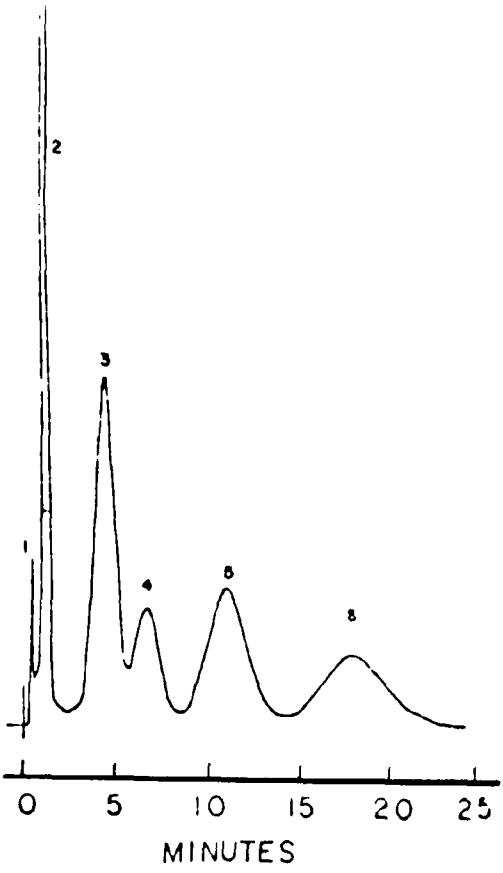


FIGURE 10
Separation of phenacyl derivatives of C3-C6 dibasic acids. Column, 25 x 0.4 cm C8 Corasil II; temperature 40°C; mobile phase: water methanol (68:32); flow rate: 3.6 ml/min; attenuation: 0.08 AUFS. (1) impurity; (2) α-bromoacetopheone; (3) malonic acid; (4) succinic acid; (5) glutaric acid; (6) adipic acid.
After Grushka, Durst and Kikta (41).

techniques, resulting in the identification of many long chain bacterial fatty acids. These fatty acids from Mycobacterium tuberculosis H37RAa were confirmed to be α-alkyl-β-hydroxy fatty acids called mycolic acids which are major components of the

cell wall. In a follow up study, Takayama and coworkers (40) had developed a HPLC procedure for the separation of C_{16}-C_{90} p-bromophenacyl esters of fatty acids by which they attempted to characterize the enzyme system involved in the biosynthesis of mycolic acid in cell free supernatant from disrupted cells of Mycobacterium tuberculosis H37Ra.

Dicarboxylic Acids

Dicarboxylic acids are important in the study of metabolic pathways and metabolic disorders. The preparation of dibasic acid derivatives is difficult (17). Grushka et al.(41) derivatized dicarboxylic acids with the crown ether procedure in good yields and separated the C_3-C_6 derivatives by a reversed phase system (Figure 10).

Isomers of Unsaturated Fatty Acids

As already shown in Figure 5, geometric and positional isomers of olefinic fatty acids were separated by solvent gradients. Pei et. al (42) have resolved geometric and positional isomers of unsaturated acids on a high efficiency reversed phase column. The isocratic separation of a series of C_{18} fatty acids with various degrees of unsaturation is shown (Figure 11). However, the cis and trans isomers of olefinic fatty acids are most selectively separated by argentation chromatography (Figure 12) (43).

Prpostaglandins

Prostaglandins and eicosanoids are widely distributed in animal and human tissues in subnanogram quantities. Although in such low concentrations, they are very effective and potent in many physilogical roles. To study the physiological effects of prostaglandins and eicosanoids, selective and sensitive analytical procedures are required. HPLC is one of a number of techniques used for the determination of low levels of prostaglandins and eicosanoids in tissues and body fluids.

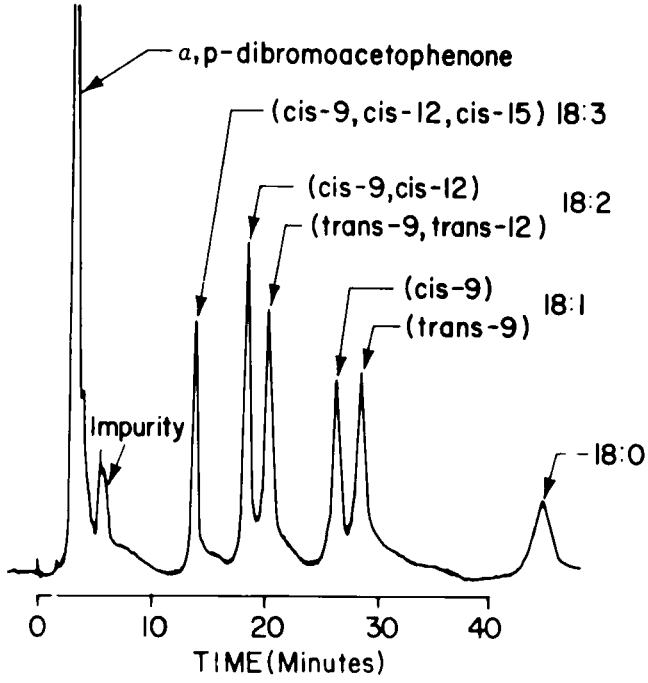


FIGURE 11
Separation of the p-bromophenacyl esters of linolenic (cis-9, cis-12, cis-15 18:3), linoleic (cis-9, cis-12 18:2), linoleaidic (trans-9, trans-12 18:2), oleic (cis-9 18:1), elaidic (trans-9 18:1), and stearic (18:0) acids. Eluent: methanol water (90:10 v/v); flow rate: 1.5 ml/min at 1000 psig; temperature: 25°C. Sample size: ca. 1.0 μg of each ester. After Pei, Kossa, Ramachandran and Heny (42).

Silica gel (29) and silver ion loaded columns (44, 45), being more selective towards structural differences within a series classification, have been used to measure families of prostaglandins. The chromatogram of a mixture of F series prostaglandin p-nitrophenacyl esters is shown (Figure 13). The normal phase chromatography gave excellent separation of the derivatives and differentiated the members of the prostaglandin F series. Efficient separation of the p-nitrophenacyl esters of prostaglandin E series is also shown (Figure 14).

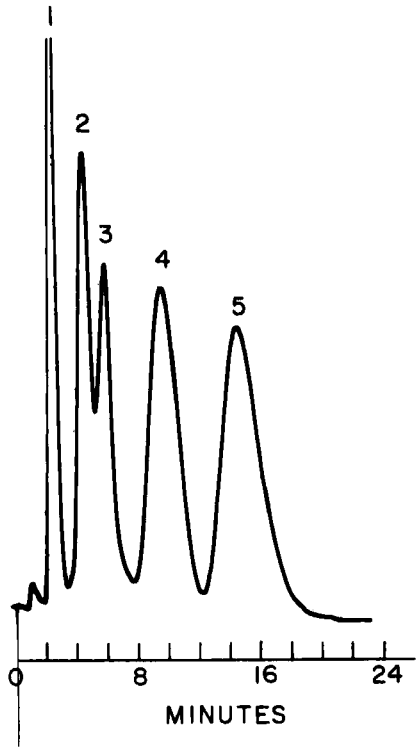


FIGURE 12
Separation of p-bromophenacyl esters of C_{18} unsaturated fatty acis on silver aluminosilicate column 25 x 0.21 cm. Mobile phase 0.01% acetonitrile in 1:13 (V/V) chloroform: hexanes, flow rate 0.57 ml/min. (1) α,p-dibromoacetophenone (2) trans vaccenic, (3) elaidic, (4) cis vaccenic, (5) oleic. After Lam and Grushka (43).

Since the difference in hydrocarbon content within a given prostaglandin series is very small, reversed phase chromatography can not achieve the refined separations that normal phase gives. However, reversed phase chromatography of p-nitrophenacyl esters are useful in the study of prostaglandin metabolism in which the metabolites being more polar are separated from the parent compounds as early eluting peaks. Fitzpatrick has applied reversed phase chromatography for the

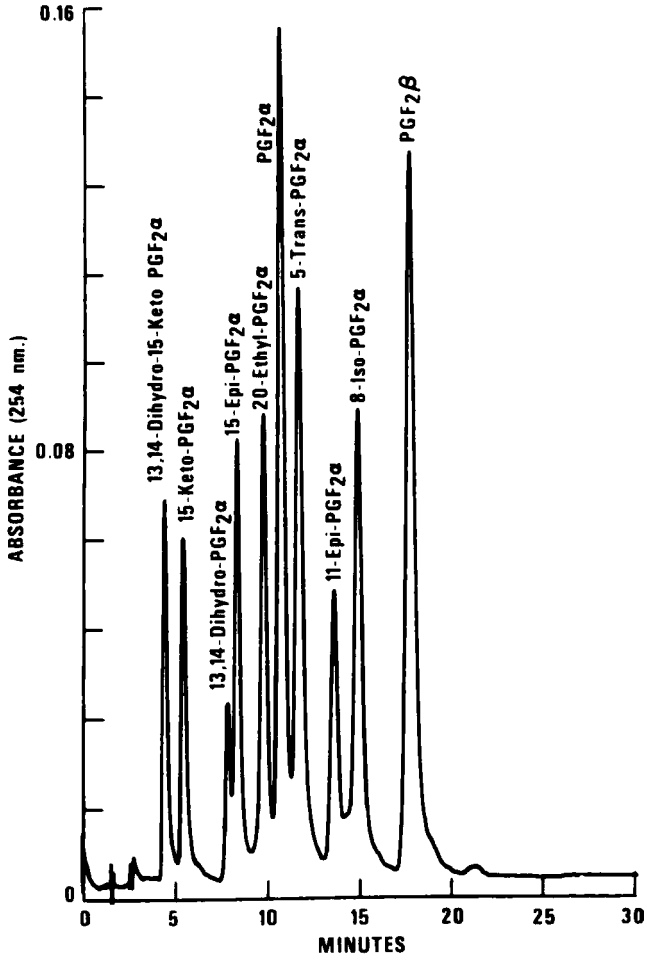


FIGURE 13
HPLC separation of a mixture of F-series prostaglandin p-nitrophenacyl esters on two series coupled 25 x 0.21 cm microparticulate silica gel columns. Mobile phase: methylene chloride-hexane-methanol (55:45:5); flow rate: 0.3 ml/min at 3000 PSI.
After Morozowich and Douglas (29).

measurement of prostaglandins and their metabolites as the p-nitrophenacyl esters in the study of enzyme activities of an

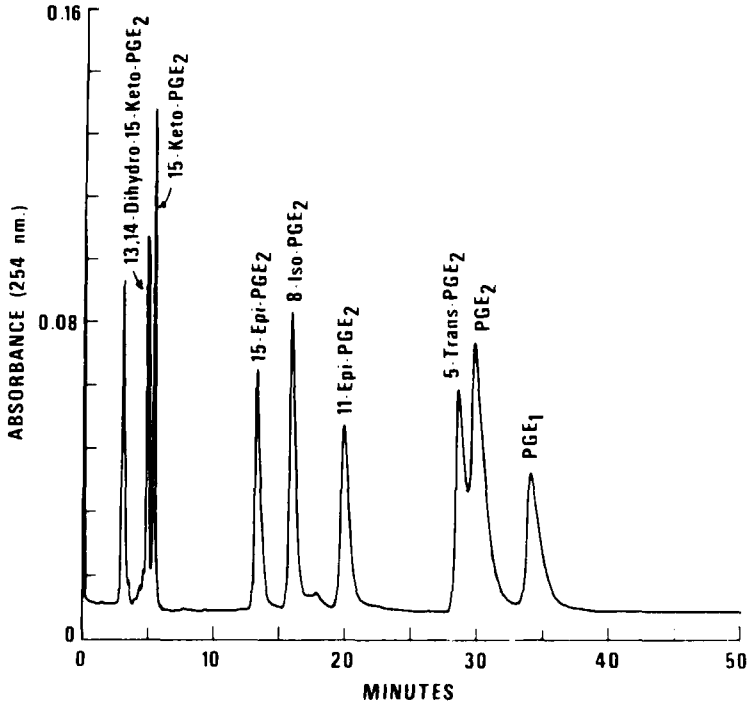


FIGURE 14
HPLC separation of a mixture of E-series prostaglandin p-nitrophenacyl esters on two series coupled 25 x 0.21 cm microparticulare silica gel columns. Mobile phase: methylene chloride-acetonitrile-dimethylformamide (160:40:1); flow rate: 0.28 ml/min at 3000 PSI.
After Morozowich and Douglas (29).

isolated prostaglandin-15-dehydrogenase (46) and a sheep seminal vesicle synthetase (31).

Recently, pancyl bromide [p-(9-anthroxyloxy) phenacyl] which possesses strong UV absorbance and fluorescence has been used to derivatize eicosanoids (47). The chromatogram of seven common eicosanoids by reversed phase HPLC is shown (Figure 15). The panacyl bromide was also applied to the measurement of E prostaglandins. Subnanogram amounts of prostaglandins were derivatized with several thousand fold excess of panacyl

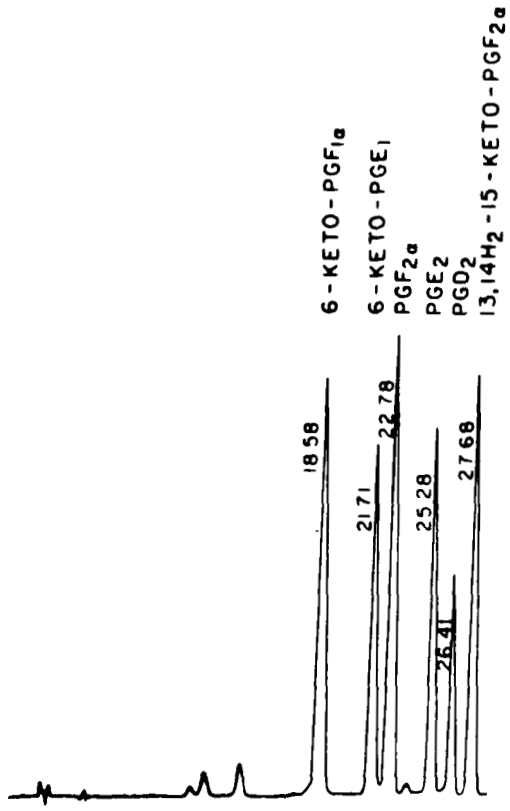


FIGURE 15
HPLC of standard eicosanoids conducted on fatty acid analysis column. Mobile phase: linear gradient of 56% to 65% acetonitrile and water over 15 minutes at a flow rate of 1.2 ml/min. AUFS was 0.05. Each eicosanoid ester is 1 to 2 ng After Watkins and Peterson (47).

bromide. Since the derivatization agent was used in such an excess, direct injection of the reaction products onto the column resulted in interference from the reagent peak and reduced sensitivity. Column switching technique was applied to transfer the postaglandins of interest in the first analytical

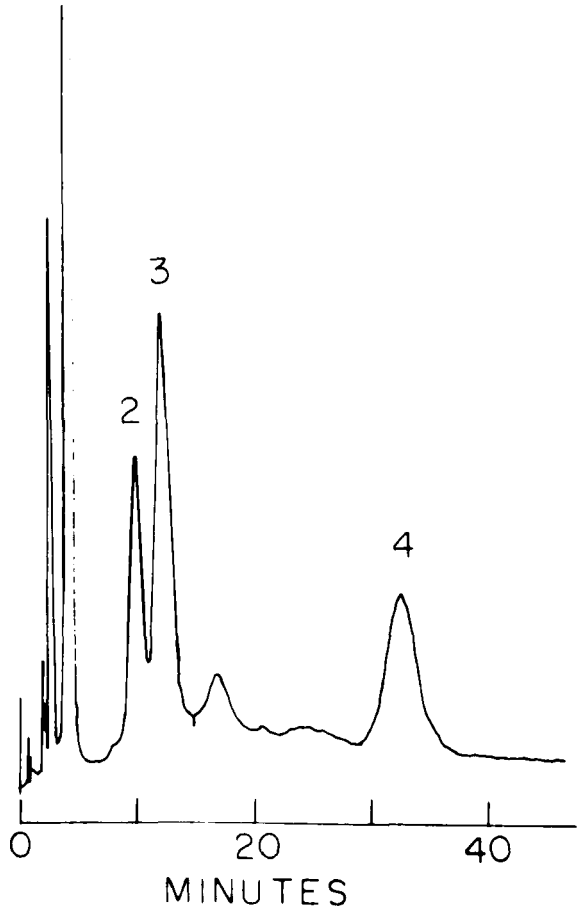


FIGURE 16
Separation of phenacyl esters of aliphatic penicillins. Mobile phase: 40% water in methanol; flow rate 2.2 ml/min. UV detection at 254 nm, 0.32 AUFS. (1) α,p-dibromoacetophenone, (2) Penicillin F, (3) Dihydropenicillin F, (4) Penicillin K. After Lam and Grushka (28).

column to the second column to eliminate interferences. Low picogram levels of E prostaglandins were determined by fluorescence (48).

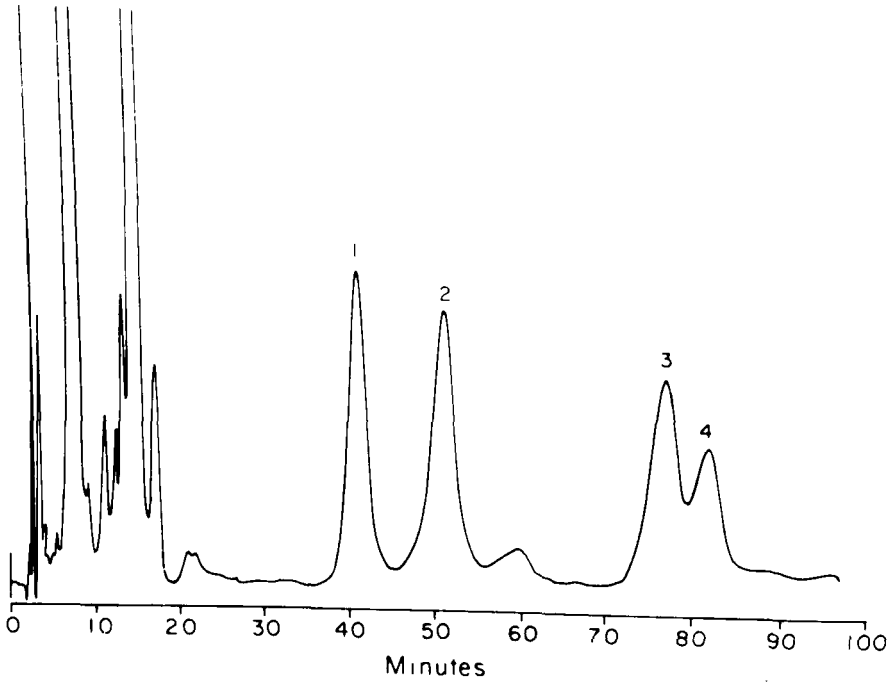


FIGURE 17
Separation of a mixture of p-bromophenacyl esters of cortolic and cortolonic acids. Two Zorbax ODS columns 25 x 0.46 cm were placed in series. Oven temperature: 40°C; solvent:methanol water (65:35) at 4500 PSI; flow rate: 1.5 ml/min. UV detection at 254 nm, 8 µl cell, 0.04 AUFS. (1) α-cortolic acid, (2) α-cortolonic acid, (3) β-cortolic acid, (4) β-cortolonic acid.
After Farhi and Monder (27).

Miscellaneous Phenacyl Esters

Some mutants of Penicillin chrysogenum on synthetic nutrients produce, in addition to the UV absorbing penicillins, several other penicillins with alkyl side chains that do not give detectable responses to the conventional fixed wavelength UV detectors. The natural alkylpenicillins, with their carboxylate moiety free, can be labeled with phenacyl halides for sensitive UV detection. The separation of three such

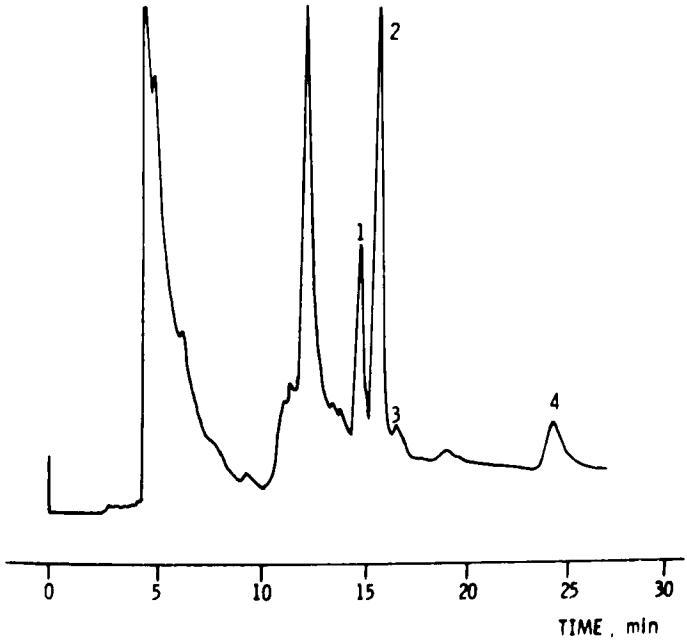


FIGURE 18
Separation of bile acid derivatives obtained from a bile sample. Column: Partisil 10/25 ODS, 25 x 0.46 cm; mobile phase: step gradient n-heptane:dioxane, 90:10 for 3 minutes, then changed to n-heptane:dioxane:2-propanol, 70:25:5; flow rate 1.2 ml/min. (1) Deoxycholic (2) chenodeoxycholic (3) ursodeoxycholic (4) cholic.
After Stellaard, Hachey and Klein (49).

phenacyl derivatives on a C_{18} column (28) is shown (Figure 16). This procedure could allow easy trapping of antibiotics from the culture media for structural and quantitative purpose.

Cortoic acids are a group of acidic metabolites of cortisol found in the urine. Determination of cortoic acid may help monitor the normal and perturbed stages of cortisol metabolism. A representative chromatogram of the cortoic acids is shown (Figure 17) (27).

Bile acids are specific for liver function. It is speculated that different liver diseases may produce varying

Table I. Detection limits of carboxylic acid derivatives at 254 nm.

Derivatives	Detection Limit	Ref
Mono-carboxylates:		
C_2 p-bromophenacyl ester	4 picomole	21
C_{20} p-bromophenacyl ester	10 picomol	21
Valproic phenacyl ester	22 picomole	26
Di-carboxylates:		
Malonic p-bromophenacyl ester	15 picomole	41
Adipic p-bromophenacyl ester	40 picomole	41
Steroidal Acid:		
Cortolonic p-bromophenacyl ester	250 picomole	27
Prostaglandin:		
PGE_2 p-nitrophenacyl ester	3 picomole	29
PGE_2 panacyl ester (fluorescence)	0.03 picomole	48
Bile Acids:		
Decxycholyl phenacyl ester	7 picomole	49
Cholyl phenacyl ester	25 picomole	49
Lithocholyl phenacyl ester	10 picomole	49
Chenodeoxycholyl phenacyl ester	10 picomole	49

amounts of conjugated and unconjugated bile acids. The assay of these bile acids in serum may thus be useful for the differential diagnosis of liver diseases. Bile acids which do not possess a chromophore can be modified with phenacyl bromides for detection (Figure 18) (49).

5-Pyrrolidone-2-carboxylic acid is a cyclic lactam of glutamic acid associated with the metabolism of glutathione via the gamma-glutamyl cycle. A procedure for determining 5-pyrrolidone-2-carboxylic acid as the 4-nitrophenacyl ester in tissue homogenates was developed (50).

SUMMARY

The phenacyl esters are sensitive, giving enhanced detection of the naturally occuring fatty acids in low

picomole concentrations as given in Table I. Phenacyl esters have been applied to the measurement of carboxylic acids including long chain and short chain fatty acids, dicarboxylic acids, hydroxyl acids, steroidal acids, penicillins and eicosanoids. Apart from the general usage in quantitative and qualitative studies, the phenacyl esters are useful in profiling of fatty acids in biological and physiological samples, and in chemotaxonomy of microorganisms, as well as in structural elucidation of bacterial fatty acids.

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