❦ Separation of Tocopherol Homologues by Selective Deacylation and Chromatography

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ABSTRACT

Isolation of tocopherol homologues can be accomplished by selective deacylation of tocopheryl esters followed by chromatographic separation of the esters from the free tocopherols.

INTRODUCTION

A recent communication by Mansson describing the deacetylation of aromatic acetates in the presence of aliphatic acetates by treatment with pyrrolidine noted that the rate of deacetylation was somewhat sensitive to steric hindrance (1). Since tocopherol esters 1, 2, 3 and 4 vary in the degree of steric bulk about the phenolic OH group, we decided to determine if tocopheryl acetates would react at rates different enough to provide a method for separating the tocopherols from each other. This would be desirable, since separation of the tocopherols is very difficult to achieve on a preparative scale either by fractional crystallization of derivatives or by chromatography.

EXPERIMENTAL

Separations

α-Tocopheryl acetate. To 20.4 g of a mixture of tocopheryl acetates (37%, α; 1.4%, β; 29.9%, γ; 25.5%, δ) dissolved in 120 mL CH_2Cl_2 under N_2 was added 10 g of pyrrolidine. After being stirred for 18 hr at room temperature, the mixture was analyzed by glpc and showed complete deacylation of the δ- and γ-acetates. The mixture was acidified with aqueous HCl, washed with H_2O, dried over $MgSO_4$, and concentrated to 21.3 g of an oil. Chromatography on Doucil adsorbent (3% acetone/hexane) gave 5.8 g of α-tocopheryl acetate, cleanly separated from β-, γ- and δ-tocopherols. Purity was determined by glpc using a 30-m DB-5 capillary column (J&W Scientific, Inc.) at 250-280 C (H_2 carrier gas).

When separation was attempted on a basic ion-exchange resin, the α-acetate was not held up and clean fractions of α-acetate were obtained. However, some hydrolysis occurred on the column and caused some free α-tocopherol to elute with γ- and δ-tocopherols.

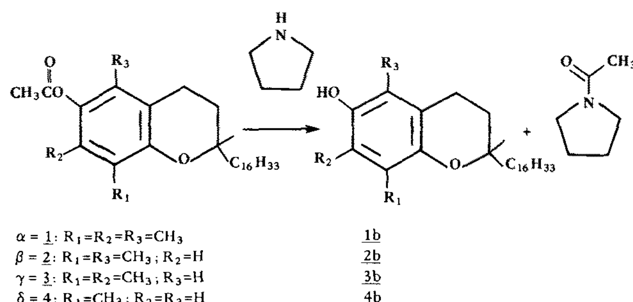
γ-Tocopherol. Fifty grams of a mixed tocopherol concentrate derived from soybean oil deodorizer distillate (5.5%, α; 0.7%, β; 25.9%, γ; 11.7%, δ; 43.8% total tocopherols) was dissolved in 250 mL of dry pyridine, and hexanoyl chloride (28.8 g, 30 mL) was added slowly at 15 C. The mixture was stirred at room temperature for 3 hr and then poured into H_2O. Extraction with ether followed by washing with aqueous HCl, water and brine, and then drying over $MgSO_4$ and evaporation gave the acylated product (71 g). A 5-g portion of this product was dissolved in 15 mL of CH_2Cl_2 and 5 mL of pyrrolidine. After the solution was stirred for 3 hr at room temperature, all the δ-hexanoate had deacylated but essentially none of the α-hexanoate and only 30% of the γ-hexanoate had reacted. The mixture was acidified with aqueous HCl and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with dilute HCl, dried over $MgSO_4$, and concentrated to 4.79 g of oil. Silica gel chromatography was conducted by first eluting the esters with 2% acetone in heptane and then eluting the free tocopherols with 5% acetone in heptane. The tocopheryl hexanoate fraction (now only γ- and α-hexanoates) was then further treated with pyrrolidine/CH_2Cl_2 to deacylate γ-tocopheryl hexanoate and then chromatographed on silica gel as above to give γ-tocopherol (96% pure, contaminated with ~2% β-tocopherol) and α-tocopheryl hexanoate. Purity was determined by glpc as described above except that d-α-tocopheryl acetate was used as internal standard.

RESULTS AND DISCUSSION

Treatment of mixed tocopheryl acetates (α, β, γ and δ) with pyrrolidine in CH_2Cl_2 at room temperature led to rapid deacylation of δ-tocopheryl acetate (~15 min). The acetates of β- and γ-tocopherol reacted more slowly (~2 hr), and α-tocopheryl acetate did not react. These rate differences are sufficient to allow selective deacetylation of β-, γ-, and δ- acetates in the presence of α-acetate. Since the polarity of the acetate is significantly different from that of the free tocopherol, this allows an efficient chromatographic isolation of α-tocopheryl acetate. In addition, if one starts with a mixture that is mainly γ- and α-tocopheryl acetates [such as is obtained after removal of δ-tocopherol from natural tocopherol concentrates with basic ion-exchange resins (2)], selective deacylation of γ-acetate 3 allows simple chromatographic purification (Doucil adsorbent, hexane/5% acetone in hexane gradient elution) of γ-tocopherol, 3b.

To make the chromatographic separation even easier, one could use longer chain esters than those shown in Scheme 1. We demonstrated that the selective deacylation does indeed proceed for the hexanoates of tocopherols, although at a slower rate than for the tocopheryl acetates. This strategy was used to prepare 96% pure γ-tocopherol. First the δ-tocopherol was removed by selective deacylation of δ-tocopheryl hexanoate followed by chromatographic separation of the δ-tocopherol from the remaining tocopheryl hexanoates. Then γ-tocopherol was isolated by selective deacylation of γ-tocopheryl hexanoate followed by chromatographic separation of γ-tocopherol from the α-tocopheryl hexanoate.

SCHEME 1



[1]All tocopherols used in this work were from natural sources so that they are all of the (2R, 4'R, 8'R)-configuration.

Thus, by appropriate choice of conditions, one can use this technique to isolate multi-gram quantities of α-, γ-, and δ-tocopherols. It should be noted, however, that the small quantity of β-tocopherol which may be present in naturally occurring mixtures can not be separated from γ-tocopherol since these isomeric tocopheryl esters have similar rates of deacylation.

A brief study of secondary amines other than pyrrolidine revealed that pyrrolidine is uniquely suited for reaction under mild conditions. Diethylamine, neat or in refluxing CH_2Cl_2 gave no deacetylation. Morpholine did not react in CH_2Cl_2 at reflux, but deacetylation of 4 in the presence of 1 was accomplished in neat morpholine at room temperature in 8 hr.

REFERENCES

1. Mansson, P., Tetrahedron Lett., 23 (17), 1845 (1982).
2. U.S. Patent 3,402,182, Eisai Co. (1968).

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The Analysis of Phospholipids in Soy Lecithin by HPLC

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ABSTRACT

A high performance liquid chromatography (HPLC) method is described for the HPLC analysis of major phospholipids in soy lecithin. The method entails dissolving soy lecithin in chloroform prior to analysis. The HPLC determination uses a normal phase column and a mobile phase of acetonitrilemethanol-H_3PO_4 with detection at 205 nm. The data presented illustrates that the method is rapid, accurate and precise for the determination of phospholipid in soy lecithin.

INTRODUCTION

Soy lecithin is a commonly used emulsifier in the manufacture of confectionery and other food stuffs (1). The surfactant behavior is influenced by the properties of the phospholipids, since each phospholipid has a different acyl chain and polar head group. Current methodology for the analysis of phospholipids is restricted to thin layer chromatography (TLC) (2) or time consuming methods based on acetone insolubles (3).

In many instances, TLC solvents could be transferred directly to HPLC. In this case, the systems used for TLC contained $CHCl_3$, which eliminated the use of direct HPLC detection since the ultraviolet (UV) cutoff for LC grade $CHCl_3$ is much greater than 205 nm. Several authors (4,5) have reported the use of various derivatives for phospholipid determination. The direct detection of these compounds is also possible using the concept of end absorption (6). The mobile phase of McCluer and Jungawala (6) consisted of 65/21/14 $CH_3CN/CH_3OH/H_2O$ on a Porasil column. It permits direct detection of phosphatidyl choline but not other phospholipids.

This mobile phase has been previously used in our laboratory (8). The other mobile phase, suggested by Hax and VanKessel (9), permitted direct detection and achieved separation but required the use of a gradient. The work of Chen and Kou (10) illustrated the separation of various phospholipids with direct detection and an isocratic mobile phase. We chose to utilize this approach for the determination of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol and phosphatidyl serine in soy lecithin.

MATERIAL AND METHODS

The HPLC used consisted of an M6000A Solvent Delivery System (Waters Associates), a Model 710B WISP (Waters Associates), a Model 720 System Controller (Waters Associates), a Model 730 Data Module (Water Associates), and a Model 165 UV Detector at 205 nm (Beckman Instruments). The HPLC Column used was a 3.9 mm × 30 cm μPorasil (Waters Associates). The HPLC mobile phase was 780/10/9 (v/v/v) $CH_3CN/CH_3OH/85\%H_3PO_4$ flowing at 2.0 mL/min.

Standards of phosphatidyl choline. phosphatidyl ethanolamine, phosphatidyl serine, and phosphatidyl inositol from soybeans were obtained from Sigma Chemical Company and dissolved in $CHCl_3$ to a final concentration of 0.1 μg/μl. Standards were kept refrigerated when not in use and prepared fresh weekly.

Samples were obtained from various manufacturers and dissolved in $CHCl_3$ prior to analysis to final concentration of 0.1 μg/μl.

RESULTS

Samples of soy lecithin containing varying amounts of the various phosphatides were analyzed, and typical compositions are shown in Table I.

Precision studies were conducted using both standard solutions and extracts of a soy lecithin. Data are summarized in Table II.

Lower limits of the various phosphatides varied from 100 ng/injection for phosphatidyl serine to 350 ng/injec-

TABLE I

Typical Phospholipid Composition of Various Soy Lecithins (% Composition)

Description	Phosphatidyl choline	Phosphatidyl ethanolamine	Phosphatidyl inositol	Phosphatidyl serine
Soy lecithin I	11.3	12.2	11.6	N.D.
Soy lecithin II	11.2	13.3	4.4	N.D.
Soy lecithin III	16.4	6.4	21.3	N.D.

N.D. = not detectable; n=2.