Fig. 2. Radial chromatograms of the isolated components and the initial streptothricins. Solvent: *n*-butanol–pyridine–acetic acid–water (15:10:3:12). Development: twice developed with the same solvent. Reagent: 0.25 % ninhydrin in ethanol. Left-hand side: AB = initial polymycin; A, B = components of polymycin; CD = initial phytobacteriomycin; C, D = components of phytobacteriomycin. Right-hand side: A–F = mixture of polymycin and antibiotic No. 4714-12 applied as a single spot; A, B = components of polymycin; C, D, E, F = components of antibiotic No. 4714-12.

From the above results it can be seen that the proposed method has considerable advantages over partition chromatography on cellulose and, we believe, may find ready use for analytical and preparative purposes.

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Preparative separation of vitamins K_1 and K_3 from vitamins $K_{2(30)}$ and $K_{2(35)}$ by column chromatography

The earlier chromatographic methods for the isolation of vitamin K compounds entailed the use of Permutit[1], silica gel[2], Decalso[3], $MgSO_4$ and $ZnCO_3$[4]. The K vitamins are destroyed during chromatography on some adsorbents, such as Decalso or alumina[5] and as a consequence, prolonged chromatography must be avoided. Differ-

ent homologues of this vitamin can be identified by reversed-phase partition chromatography on silicone-coated paper[6]. MARTIUS[7] has isolated various forms of the K vitamins from extracts of animal tissue using the counter-current distribution technique.

Advances in silicic acid chromatography have facilitated the resolution of lipid mixtures and isolations of their components[8,9]. In the work to be presented, the authors have applied this method to the separation and isolation in a synthetic mixture of vitamin K_1(2-methyl-3-phytyl-1,4-naphthoquinone or phylloquinone), vitamin $K_{2(30)}$ (2-methyl-3-farnesylfarnesyl-1,4-naphthoquinone), vitamin $K_{2(35)}$ (2-methyl-3-farnesylgeranylgeranyl-1,4-naphthoquinone) and vitamin K_3 (2-methyl-1,4-naphthoquinone).

The solvent used in these experiments was either spectroanalyzed analytical grade *n*-hexane or redistilled petroleum ether (b.p. 60–70°). All other reagents were redistilled in glass with the exception of ethyl ether which was U.S.P. specification. Paper chromatography was carried out on silicone-coated Whatman No. 1 filter paper as described by GREEN AND DAM[6].

The preparation of the chromatographic column was similar to that described by HIRSH AND AHRENS[8]. The silicic acid was washed with 25 ml of ethyl ether, 50 ml of acetone–ethyl ether (1:1, v/v) and finally 40 ml of ethyl ether. Following the completion of these washes, the column was equilibrated for 16 h with *n*-hexane. During the pretreatment and fractionation procedures, the jacketed chromatographic apparatus was maintained at a constant temperature of 25°. The amounts used in the chromatographic runs ranged from 30 to 97 mg. Preliminary adsorption isotherm studies indicated that the K vitamins could be obtained in micro quantities.

The first chromatographic elution scheme (called scheme 1) was started with 1 % ethyl ether in *n*-hexane followed by two subsequent stepwise developing solutions. These were a 4 % solution of ethyl ether in *n*-hexane and 100 % ethyl ether which was employed to clear the column. However, in order to obtain better resolution of the four-component mixture, a second scheme (called scheme 2) was employed by starting with a concentration of 0.5 % ethyl ether in *n*-hexane followed by 1 % and 4 % ethyl ether in *n*-hexane respectively. Again, 100 % ethyl ether was used to clear the column.

The eluted fractions were collected in 25 ml volumes at a flow rate of approximately 0.5 ml/min. Immediately after elution from the column, ultra-violet spectroanalysis was performed on a Beckman DK-2 recording spectrophotometer. Subsequently, the solvent was evaporated from the tubes at 50° using air jets and the weight of each fraction determined gravimetrically. The residues were examined by infra red spectrophotometry on a Perkin Elmer infra-red apparatus model 21 for further characterization.

When vitamins K_1, $K_{2(30)}$, $K_{2(35)}$ and K_3 were chromatographed together, employing scheme 1, 98 % of the total mixture was recovered but the first peak was heterogeneous and was produced by vitamins K_1, $K_{2(30)}$, $K_{2(35)}$, as determined by reversed-phase partition chromatography on silicone-coated paper. Ultra-violet spectroanalysis and partition chromatography revealed that the second peak was caused by vitamin K_3.

An increased resolution of the four-component mixture was accomplished employing scheme 2 and this is shown in Fig. 1. Reversed-phase partition chromatog-

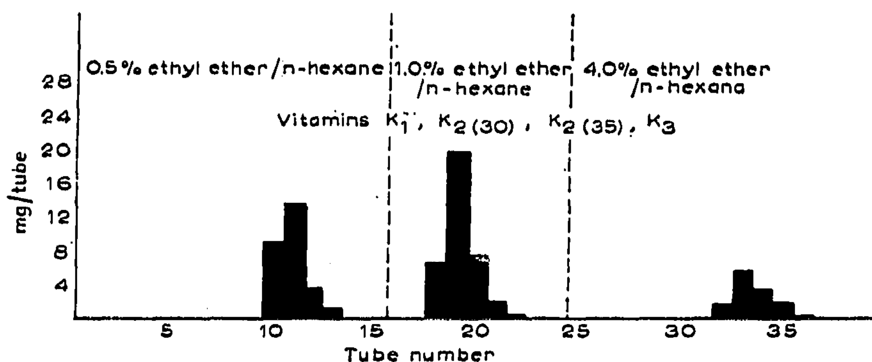

Fig. 1. Chromatography of vitamins K_1, $K_{2(30)}$, $K_{2(35)}$ and K_3 employing elution scheme 2: mg/tube *versus* tube number. First peak: vitamin K_1; second peak: vitamins $K_{2(30)}$ and $K_{2(35)}$; third peak: vitamin K_3.

raphy indicated that the first peak, eluted with 0.5 % ethyl ether in *n*-hexane, was homogeneous and was phylloquinone. The second peak, eluted with a 1.0 % solution was a mixture of vitamin $K_{2(30)}$ and $K_{2(35)}$, with the former more concentrated in the front of the peak and the latter more concentrated in the tail of the peak. The third peak consisted solely of menadione. Infra-red analyses supported these conclusions. So far, the employment of gradient elution has failed to improve the resolution of this four-component system.

It appears, therefore, that the procedure described is useful for the separation of vitamins K_1 and K_3 as homogeneous fractions from the two vitamins, $K_{2(30)}$ and $K_{2(35)}$, and shows promise for the isolations of these substances from a lipid mixture isolated from tissues and body fluids. It must be kept in mind, however, that these K homologues may behave differently in the presence of other lipids. Such studies are currently being undertaken both with synthetic lipid mixtures and lipid extracts of biological material.

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