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Note

Separation of prenylquinones, prenylvitamins and prenols on thin-layer plates impregnated with silver nitrate

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The class of prenyllipids (isoprenoid lipids) can be divided into two groups. The pure prenyllipids, the carbon skeleton of which is built up solely from isoprene units, consist of carotenoids, prenols and sterols, including vitamin A and D[1–4]. The mixed prenyllipids contain an isoprenoid side-chain (prenyl chain), which is bound to a non-isoprenoid aromatic nucleus, a benzoquinone or a naphthoquinone ring in the case of prenylquinones and a porphyrin ring for the chlorophylls. To this group also belong the prenylvitamins K_1 and K_2, which are prenylnaphthoquinones, and the E vitamins (tocopherols), which represent cyclic forms (chromanols) of reduced prenylbenzoquinones[1–5].

Most of the prenyllipids, such as chlorophylls, carotenoids and prenylquinones, and also tocopherols and vitamin K_1, which occur in plant lipid extracts can be separated by thin-layer chromatography (TLC) using silica gel plates or special mixtures of silica gel with other adsorbents[2,6–8]. With prenols and mixed prenyllipids there exist compounds with one double bond per isoprene unit and others with partially or fully unsaturated isoprenoid chains. These prenyllipids have almost identical R_F values on adsorption TLC, and cannot be separated on silica gel plates[6,8].

There are many organic compounds with electron-donor properties that can form weak charge-transfer complexes with suitable π-complexing metals such as silver, a property which can be used in chromatographic separation processes[9], using liquid, thin-layer and high-performance liquid chromatography[9–14]. The argentation silica gel method (impregnation of plates with silver nitrate) has proved to be a useful technique for the separation of various types of lipophilic unsaturated organic compounds including olefins, fatty acid methyl esters, *cis*- and *trans*-monoenic esters and sesquiterpene alcohols[9–15]. This technique has recently been applied to the separation of phytylpheophytin a from geranylgeranylpheophytin[16] but has not been applied to prenylquinones or other prenyllipids. Whether the silver nitrate silica gel technique can also be applied to the separation of prenylquinones and other natural prenyllipids

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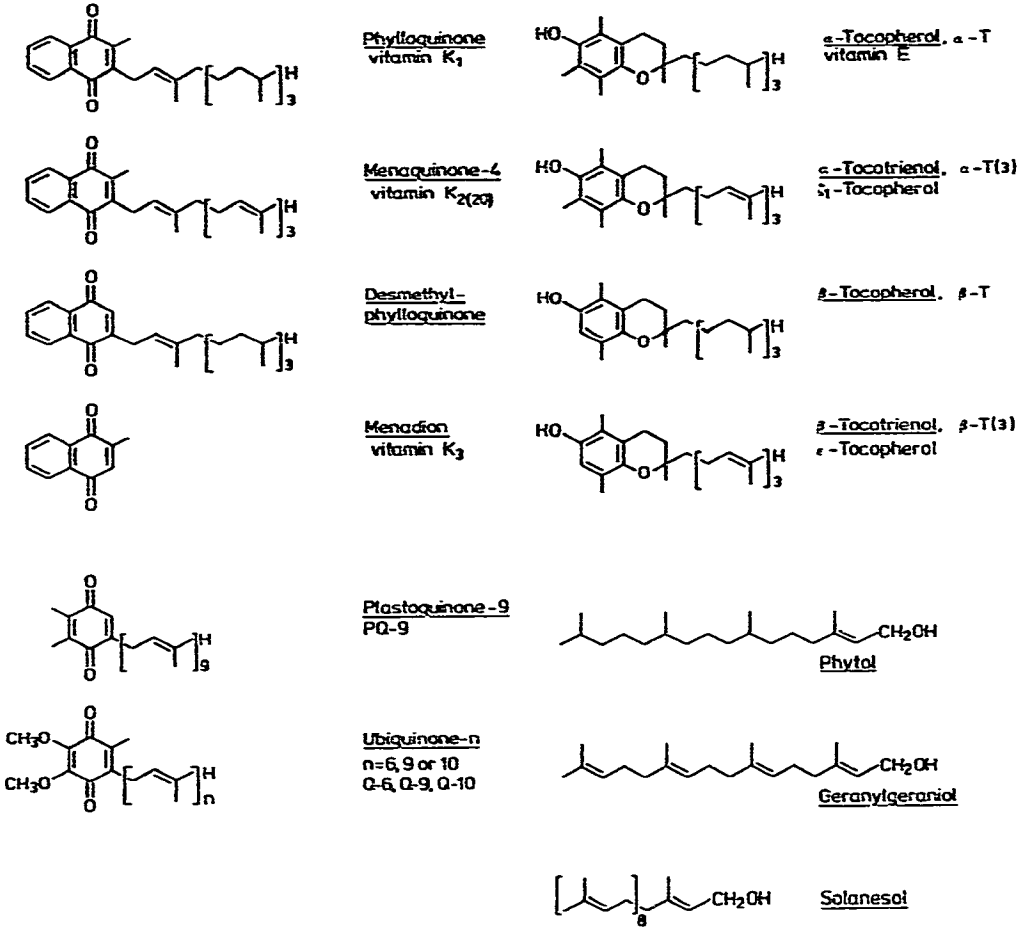

Fig. 1. Structures of prenylquinones, prenols and prenylvitamins.

which differ in the number of double bonds (such as phylloquinone (K_1) from menaquinone-4, tocopherols from the corresponding tocotrienols and phytol from geranylgeraniol) was not known and has been investigated in this work. The structures of these prenyllipids are shown in Fig. 1.

EXPERIMENTAL

Commerical or prefabricated silica gel plates were impregnated with silver nitrate in a darkened room by dipping the plate in freshly prepared 5% silver nitrate solution. The plates were dried for 1–2 h in the dark in an oven (95°C) and stored in a desiccator until used. To obtain a good resolution the plates were prepared freshly on the day of use. Detection was carried out by spraying the chromatogram with a 0.05% solution of rhodamine B in ethanol and viewing in long-wavelength UV light. Prenylquinones appear as violet bands and prenols and sterols as faint pink spots on a yellow background. Spraying with 0.2% anilinonaphthalenesulphonic acid in metha-

nol was also found useful. With tocopherols and tocotrienols, spraying with Emmerie–Engel reagent[17] was also applied (red spots).

RESULTS AND DISCUSSION

Adsorption chromatography on silica gel plates separates prenylquinones from β-carotene and prenols (phytol, geranylgeraniol), as has been shown previously[2,4] (Fig. 2A). Compounds with the same carbon skeleton that differ only in three double bonds appear on silica gel TLC plates as one band, *e.g.*, phylloquinone K_1 and menaquinone-4 (MK-4), α-tocopherol and α-tocotrienol, and phytol and geranylgeraniol, as is demonstrated in Fig. 2A. Prenylquinone homologues with side-chains of different lengths cannot be separated on silica gel plates by adsorption chromatography. Thus ubiquinone-10, which contains an additional isoprene unit, exhibits a similar R_F value to ubiquinone-9 (Fig. 2A).

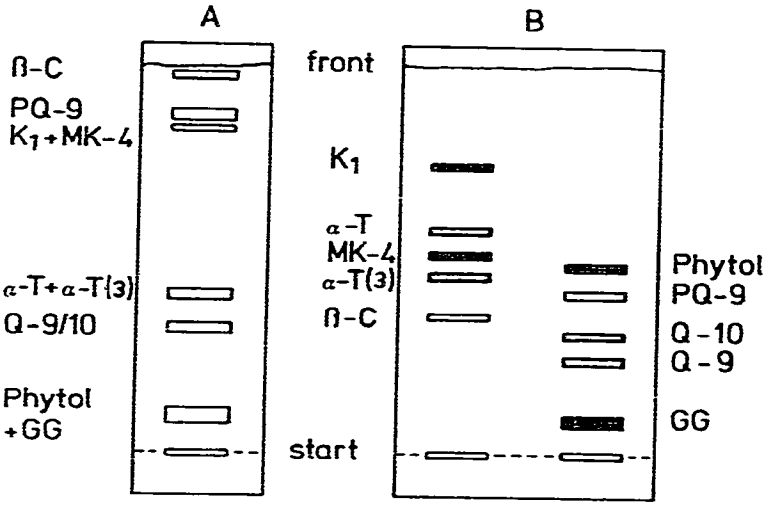

Fig. 2. Thin-layer chromatography of prenylquinones and prenols. Adsorption chromatography on (A) silica gel plates [solvent: light petroleum (b.p. 50–70°C)–diethyl ether (9:1)], and (B) on silica gel plates impregnated with silver nitrate [solvent: light petroleum (b.p. 50–70°C)–chloroform–acetone 50:10:17)]. K_1 = phylloquinone, vitamin K_1; MK-4 = menaquinone-4; PQ-9 = plastoquinone-9; Q-9, Q-10 = ubiquinone-9 and -10; α-T = α-tocopherol; α-T(3) = α-tocotrienol; β-C = β-carotene; GG = geranylgeraniol.

On silver nitrate-impregnated silica gel plates the chromatographic mobility of these prenyllipids is, however, completely changed. Phylloquinone K_1, the phytylmenadione, with only one double bond in the side-chain, moves far ahead of menaquinone-4, the geranylgeranylmenadione, which has four double bonds in the side-chain. This also applies to other prenyllipids. α-Tocopherol (no double bonds in the side-chain) has a higher R_F value than α-tocotrienol (three double bonds in the isoprenoid chain). Correspondingly, phytol has a much higher mobility than geranylgeraniol. There is also a separation of the ubiquinone homologues Q-9 and Q-10. In contrast to pure silica gel, the mobility of β-carotene and plastoquinone is much lower on the silver nitrate-impregnated silica gel plate (Fig. 2B).

Several other solvent systems were applied in order to obtain more information on the specific chromatographic behaviour of pure and mixed prenyllipids on silver nitrate-impregnated silica gel plates (Table I). In all instances the components of the same carbon skeleton with fewer double bonds migrate further than those with three double bonds more: K_1 before MK-4, α-T before α-T(3), β-T before β-T(3) and phytol before geranylgeraniol. It is of interest that the methylnaphthoquinone menadione (K_3), which has no isoprenoid side-chain, moves almost as far as its geranylgeranylderivative menaquinone-4. Prenyllipids with one methyl group less migrate close behind their methyl derivatives, *e.g.*, desmethylvitamin K_1 behind K_1, β-tocopherol behind α-tocopherol and β-tocotrienol behind α-tocotrienol (Table I).

Ubiquinone homologues possess the same mobility on the regular adsorption silica gel plates. On the silver nitrate-impregnated-plates additional double bonds in

TABLE I

SEPARATION OF PRENYLQUINONES, PRENOLS AND PRENYLVITAMINS ON SILVER NITRATE-IMPREGNATED SILICA GEL PLATES USING DIFFERENT SOLVENT SYSTEMS

Prenyllipid	$R_F \times 100$*				
	S1	*S2*	*S3*	*S4*	*S5*
β-Carotene (provitamin A)	7	10	64	35	–
Phytol	42	55	56	50	48
Geranylgeraniol	10	17	16	12	10
Solanesol	9	16	14	10	–
α-Tocopherol	60	70	68	56	54
α-Tocotrienol (ζ-tocopherol)	48	58	55	45	–
β-Tocopherol	58	69	64	52	–
β-Tocotrienol (ε-tocopherol)	46	55	49	40	–
Vitamin K_1 (phylloquinone)	67	71	76	73	70
Vitamin $K_{2\,(20)}$ (menaquinone-4)	50	63	62	50	49
Desmethylvitamin K_1	63	70	73	63	–
Vitamin K_3 (menadion)	48	57	56	47	46
Plastoquinone-9	18	31	50	41	–
Ubiquinone-6	25	42	48	40	–
Ubiquinone-9	5	21	35	24	–
Ubiquinone-10	9	28	38	30	–
α-Tocoquinone	–	–	–	59	50
Vitamin A alcohol, vitamin A aldehyde	–	–	–	62	51
Vitamin A palmitate	–	–	–	82	77
Vitamin D_2	–	–	–	32	19
Vitamin D_3	–	–	–	39	25
Ergosterol	–	–	–	28	15
Cholesterol	–	–	–	41	29
β-Sitosterol	–	–	–	42	30

* Solvents:
S1 = hexane–ethyl acetate–diisopropyl ether (2:1:2);
S2 = hexane–ethyl acetate–diisopropyl ether (2:2:1);
S3 = light petroleum (b.p. 50–70°C)–chloroform–acetone (50:10:24);
S4 = light petroleum (b.p. 50–70°C)–chloroform–acetone (50:10:17);
S5 = hexane–ethyl acetate–diisopropyl ether (2:1:1).

the side-chain generally lead to a decrease in the R_F values. Thus ubiquinone-9 with three double bonds (+ three isoprene units) more, moves far behind ubiquinone-6. Ubiquinone-10, however, moves ahead of ubiquinone-9 in all solvent systems used; this indicates that an increase in the chain length by another isoprene unit gives a stronger tendency for an increase in the R_F value than the tendency for a decrease due to the additional double bond. It is of interest in this respect that a slight increase in the R_F value of Q-10 compared with Q-9 can also be seen on silica gel plates that have not been treated with silver nitrate. This demonstrates that in silver nitrate-impregnated silica gel plates the adsorption chromatographic separation principles of normal untreated silica gel plates are working also. With this point in mind, one has also to consider the R_F value of solanesol, which on the silver nitrate-impregnated plates is only slightly lower than that of geranylgeraniol. Solanesol, a C_{45}-prenol, has five isoprenoid units and five double bonds more than the C_{20}-prenol geranylgeraniol. The extra five isoprene units tend to increase the R_F value of solanesol, but the extra five double bonds decrease it below that of geranylgeraniol.

The use of the silver nitrate TLC method was also investigated for the separation of sterols and prenylvitamins. Cholesterol however, cannot be separated from sitosterol; both sterols possess one double bond and exhibit a similar mobility, which is higher than that of ergosterol with three double bonds. Vitamin D_2, which in the same carbon skeleton contains one double bond more than vitamin D_3, moves as expected behind vitamin D_3. Vitamin A aldehyde and vitamin A alcohol are not separated; vitamin A palmitate, in turn, has a high mobility.

With prenols, prenylquinones and prenylvitamins the separation sequence is the same in all five solvent systems, except for β-carotene, the provitamin A with eleven double bonds. In the first two solvent systems it migrates very little and remains not only below plastoquinone-9, but also below geranylgeraniol. In the more polar solvent systems, which contain chloroform and acetone, it exhibits a higher mobility. With solvent 4, β-carotene runs between geranylgeraniol and plastoquinone-9; in solvent 3 it moves above plastoquinone-9 and even further than phytol (Table I). This chromatographic behaviour of β-carotene indicates that in the silver nitrate-impregnated silica gel TLC system two principles are working. With less polar solvent systems the R_F value of β-carotene is lowered by the silver ions because of its eleven double bonds, and with more polar solvents the chromatographic mobility increases; the very high R_F values of the untreated silica gel plate, where β-carotene migrates behind the solvent front (Fig. 2A), are, however, not reached.

The results of this investigation indicate that the silver nitrate-impregnated silica gel techniques can be applied successfully for the separation and purification of prenylquinones, prenols and prenylvitamins that differ in their number of double bonds. Compared with normal silica gel plates silver ions decrease the R_F value of prenyllipids with more double bonds; a longer side-chain will, however, increase the R_F values. The final R_F value found for a certain substance is then the combined effect of these two opposing principles.

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