TABLE II

$R_F \times 100$ VALUES ON NEUTRAL ALUMINA ADSORBENT

Compound	$R_F \times 100$								
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9
Triphenyl phosphate	93	73	0	36	94	90	35	90	91
Triphenyl phosphite	92	70	17	—	—	—	—	—	—
Triphenylphosphine oxide	85	53	0	0	68	13	65	84	75
Triphenylphosphine	95	74	54	93	94	91	91	92	92
Tricresyl phosphate	90	70	0	32	88	88	91	93	91
Triphenylarsine	94	78	75	93	91	94	90	93	94

For the solvent systems see Table I.

TABLE III

$R_F \times 100$ VALUES OF TRIPHENYLPHOSPHINE OXIDE ON VARIOUS ADSORBENTS WITH ACETONE SOLVENT

Adsorbent	$R_F \times 100$
Silica gel G	87
Silica gel G (activated at 160°)	81
Silica gel + 5% gypsum	82
Silica gel + 3% starch	76
Alumina + 3% starch	80
Silica gel–alumina–gypsum (14:14:2)	

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Separation of vitamin D esters by thin-layer chromatography*

There is evidence that vitamin D, like other sterols[1,2], is esterified during intestinal absorption[3]. The nature of the esters thus formed is currently being investigated in this laboratory with ^{14}C vitamin D_3 in rats. This communication describes methods for separating esters of vitamins D_2 and D_3 with thin-layer chromatography.

Methods

Esters were prepared by a modification of the method of KUKSIS AND BEVERIDGE[4] either from commercially available fatty acid chlorides (butyryl, hexanoyl,

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octanoyl, decanoyl, lauroyl and oleoyl, J. T. Baker Chemical Co., and myristoyl, palmitoyl and steroyl, Matheson Scientific, Inc.) or from acid chlorides synthesized from fatty acids (linoleic, linolenic and arachidonic, Sigma Chemical Co.) and oxalyl chloride (Eastman Organic Chemicals).

To synthesize the acid chlorides, 0.58 mmole fatty acid was dissolved in 3.5 ml petroleum ether and refluxed for one hour with 1.45 mmole oxalyl chloride. Excess oxalyl chloride was removed after cooling by extracting with 0.5 ml water. To synthesize the esters, 0.4 mmole vitamin D_2 or D_3 (Mann Research Laboratories, Inc.) was refluxed with 60 ml benzene to which 0.58 mmole acid chloride and 0.05 ml pyridine had been added. The solvent was removed under vacuum and the residue was chromatographed in the dark under N_2 on 20 × 20 cm plates covered with Silica Gel H (Brinkman Instruments) in the system hexane–benzene 1:1. The esters were visible under U.V. light as a wide band near the solvent front and were eluted with methylene chloride and stored in the dark under N_2 at 4°. A more detailed description of the synthesis and the physical characteristics of the esters will be published elsewhere.

Thin layers 250 μ thick were prepared on 20 × 20 cm plates from a slurry of Silica Gel H (Brinkman Instruments) and 55 ml 0.02 % Rhodamine G in distilled water. For separation of the unsaturated fatty acid esters, 20 g Silica Gel G (Brinkman Instruments) made up with 50 ml 1.4 % silver nitrate ($AgNO_3$) and 0.02 % Rhodamine G in distilled water was used.

Results

The results are shown in Tables I and II.

The mobility of the esters was greater when the proportion of benzene in the solvent system was increased (Table I). The R_F values were found to be dependent upon the chain length of, as well as the number of unsaturated double bonds in, the fatty acid (Table I).

TABLE I

R_F VALUES* FOR ESTERS OF VITAMINS D_2 AND D_3 CHROMATOGRAPHED ON SILICA GEL IN HEXANE–BENZENE 1:1 (A) AND 1:2 (B)

Ester	*A*		*B*	
	D_2	D_3	D_2	D_3
Acetate	0.15	0.21	0.26	0.29
Butyrate	0.29	0.29	0.38	0.41
Hexanoic	0.36	0.35	0.50	0.51
Octanoic	0.44	0.40	0.58	0.58
Decanoic	0.46	0.42	0.65	0.60
Laurate	0.47	0.47	0.67	0.65
Myristate	0.49	0.49	0.71	0.69
Palmitate	0.52	0.54	0.73	0.73
Stearate	0.55	0.55	0.75	0.73
Oleate	0.52	0.53	0.73	0.70
Linolate	0.48	0.48	0.71	0.69
Linolenate	0.43	0.42	0.61	0.64
Arachidonate	0.43	0.43	0.69	0.66

* Mean of three or four determinations.

The esters were more clearly separated on the basis of the degree of unsaturation of the fatty acid when chromatographed in silica gel impregnated with $AgNO_3$ (Table II).

There was essentially no difference in mobility of the same esters of vitamins D_2 and D_3 (Tables I and II).

TABLE II

R_F VALUES FOR ESTERS OF VITAMINS D_2 AND D_3 CHROMATOGRAPHED ON SILICA GEL IMPREGNATED WITH $AgNO_3$ IN HEXANE–BENZENE 1:2

Ester	D_2	D_3
Stearate	0.79	0.78
Oleate	0.57	0.58
Linolate	0.41	0.41
Linolenate	0.30	0.30
Arachidonate	0.18	0.20

Discussion

The results show that the migration of vitamin D esters on silica gel is dependent on the chain length as well as the degree of unsaturation of the fatty acid moiety, the longer chain length increasing, and the greater degree of unsaturation decreasing, mobility (Table I). These findings are thus similar to those obtained with thin-layer chromatography of esters of cholesterol[5,6] and of β-sitosterol[7]. The effect of unsaturation gives rise to "critical pairs" of esters with the same rates of migration: palmitate–oleate, myristate–linolate and laurate–linolenate (Table I).

The unsaturated esters can be separated from the saturated ones with longer chain lengths on the basis of the number of unsaturated double bonds by thin-layer chromatography on silica gel impregnated with $AgNO_3$ (Table II). This method has previously been used to fractionate cholesterol esters in the same manner with much the same results[8,9].

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