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Note

Rationalisation of the chromatographic behaviour of vitamin D_2/D_3 and related compounds in adsorption high-performance liquid chromatography

D. THORBURN BURNS

Department of Analytical Chemistry, The Queen's University of Belfast, Belfast, BT9 5 AG (Northern Ireland)

CHRISTEEN MacKAY

Department of Chemistry, Loughborough University, Loughborough, Leics. (Great Britain)

and

J. TILLMAN

Fisons Limited, Pharmaceutical Division, Research and Development Laboratories, Bakewell Road, Loughborough, Leics. LE11 0QY (Great Britain)

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The concept of molecular planarity has been used in order to explain the relative retention of compounds in both classical column and thin-layer chromatography when the adsorbent used as stationary phase is silica or alumina. In particular, silica is effective for the separation of isomeric compounds; the surface of the adsorbent is regarded as being approximately planar and therefore, molecules which are planar or can readily adopt a planar configuration will fit more readily on to the adsorbent surface sites compared with isomeric molecules which adopt a less planar or non-planar configuration[1]. This concept does not seem to have been discussed in relation to the separation of compounds by high-performance liquid chromatography (HPLC) but it is clearly applicable.

Vitamin D and its structurally related thermal and photo isomers provide an interesting set of stereochemically related compounds[2] for which a correlation of the elution order and molecular planarity can be derived. Vitamin D_2 and vitamin D_3 have almost identical molecular structures[3]; the structural difference is in the side chain R. Vitamin D_2 has a double bond in the $C_{22}C_{23}$ position and a methyl group on C_{24}; vitamin D_3 does not have these features. The chromatographic behaviour of vitamin D_2 and vitamin D_3 is similar[4].

In the determination of vitamin D_2 or vitamin D_3 in pharmaceutical preparations, the thermal isomer, pre-vitamin D, is important in ascribing biological potency to a product[5]. The photo isomer of pre-vitamin D, tachysterol, is important in the impurity screening of the product[6]. A suitable quantitative procedure for the assay of vitamin D_2 in multivitamin tablets has been reported[7]. The elution order of pre-vitamin D, vitamin D and pro-vitamine D was similar to the results obtained by other workers applying adsorption mode of chromatography to the separation of the thermal and photo isomers of vitamin D[8–11]. In this communication, a rationalisation of the elution order of the vitamin D compounds is derived from a consideration of

TABLE I

RETENTION DATA FOR VITAMIN D_2/D_3 AND RELATED COMPOUNDS

n.a. = Not available.

Author	D_2/D_3	*Adsorbent*	*Solvent*	*Retention times (min)*				
				Pre-vitamin D	*Lumisterol*	*Tachipterol*	*Vitamin D*	*Pro-vitamin D*
				Increasing order of planarity →				
Steuerle[8]	D_3	Al_2O_3	Chloroform	7.8	8.5	11.0	13.2	19.5
Tartivita *et al.*[9]	D_2/D_3	SiO_2	Chloroform–*n*-hexane–tetrahydrofuran (70:30:1)	10.1	11.0	11.5	17.1	24.1
Hoftsas *et al.*[10]	D_3	SiO_2	Isooctane–ethanol (98.4:1.6)	7.4	8.2	9.8	10.5	12.0
Vanhaelen-Fastré and Vanhaelen[11]	D_2/D_3	SiO_2	Light petroleum (b.p. 40–60°)–1,2-dichloroethane–dioxane (90:8:2)	9.1	10.3	15.8	14.8	22.0
Mackay *et al.*[7]	D_3	SiO_2	Cyclohexane–isopropanol (98.75:1.25)	15	n.a.	n.a.	40	58

the relative planarity of the molecules and the stereochemical conformations adopted by them in solution[12,13].

The classical configuration of the vitamin D compounds are illustrated in Fig. 1. The planarity order of the compounds of interest and the observed retention time are listed in Table I.

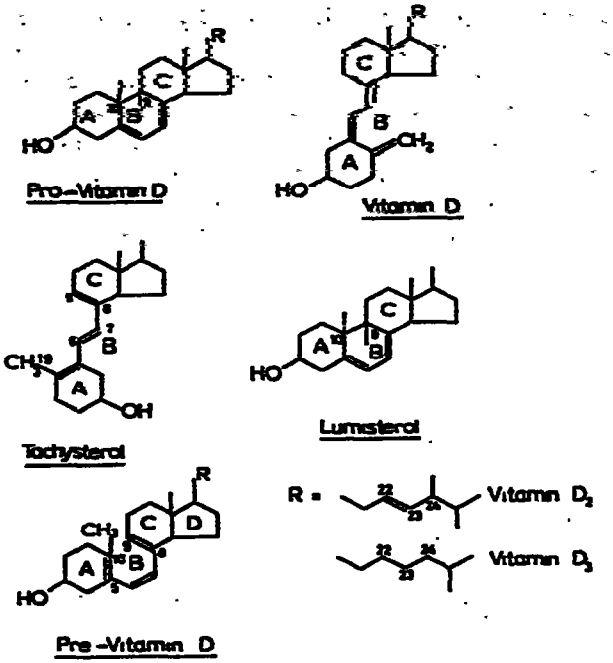

Fig. 1. Classical configurations of the vitamin D compounds.

The important features of the stereo configurations of the molecules are as follows.

Pro-vitamin D. Rings A, B and C are in a chair conformation and the overall molecule is planar.

Vitamin D. Ring A is in a fixed chair conformation, ring B is open and ring C is in a chair conformation. The overall molecular conformation is thus less planar than the pro-vitamin D.

Tachysterol (6:7, *trans* isomer of pre-vitamin D). Ring A is in a fixed chair conformation which is more flexible than ring A in the vitamin D molecule. Ring B is open and ring C is in a strained chair conformation because of the double bond in C_8C_9 position. Furthermore, there is interaction between the hydrogen atoms on C_6 and C_{19}. The overall molecular conformation is therefore less planar than vitamin D.

Lumisterol (9:10 *anti* isomer of pro-vitamin D). Ring A is in a chair conformation, ring B is in a fixed chair conformation and ring C is in a boat conformation because of the 9β, 10α configuration. The net result is a relatively non-planar molecule less planar than tachysterol but more planar than pre-vitamin D.

Pre-vitamin D. Ring A is in a strained chair conformation because of the double

bond in the C_5C_{10} position. Ring B is open and ring C is in a strained chair conformation because of the double bond in the C_8C_9 position. Furthermore, ring A is below the plane of rings C and D and the overall result is an essentially non-planar molecule.

The relative planarity order of the vitamin D related compounds is thus: pro-vitamin D, vitamin D, tachysterol, lumisterol and pre-vitamin D which correlates inversely with the elution order of these compounds as shown by their retention times in Table I. The reverse order of tachysterol and vitamin D, noted by one pair of workers may be accounted for by a slight conformational change induced by interaction with the solvent; any acidic species in the solvent could effect a change in molecular conformation. The concept of planarity and its relation to elution order reported here would be of general application to the interpretation of the relative retention of any series of structurally related isomeric compounds, which contained the same functional group in HPLC, adsorption mode of chromatography. It would not necessarily apply if the series of compounds contained functional groups of different polarity.

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