STUDIES ON THE CHEMICAL ISOMERIZATION OF VITAMIN D

IV. IDENTIFICATION OF VITAMIN D₂ AND ITS RELATED COMPOUNDS BY THIN LAYER CHROMATOGRAPHY

TADASHI KOBAYASHI¹

*National Institute of Hygienic Sciences, Osaka Branch
Hoenzaka-cho, Higashi-ku, Osaka*

(Received February 18, 1967)

In the previous reports (1, 2) a new identification method for vitamin D₂ isomers using gas chromatographic and alumina column chromatographic separations was described. In this paper thin layer chromatographic separations of the mixtures of vitamin D₂ and its related compounds were investigated with the same object. If vitamin D₂ isomers are readily separated one another by a thin layer chromatography, it will be more useful for the identification of vitamin D₂ isomers because of its simplicity.

There have been a few reports (3-5) on the thin layer chromatography of vitamin D, but they were not tried on the separation of the mixtures of vitamin D isomers. Norman *et al.* (6) reported on the separation of irradiation mixtures of ergosterol by a thin layer chromatography using Cab-O-Sil. silica gel as an adsorbent and 10 % acetone in Skellysolve B as a developing solvent. They obtained six spots in the chromatogram and confirmed that three spots of them were due to vitamin D₂, ergosterol and tachysterol₂.

The author reported previously on the separation between *cis* and *trans* isomers of vitamin A by thin layer chromatography, which was a partially revised method of that reported by Planta *et al.* (7), using Kieselgel G as an adsorbent and *n*-hexane methyl ethyl ketone (11:2) as a developing solvent (8). As this method was expected to be useful for the separation of *cis* and *trans* isomers of vitamin D₂, it was applied to vitamin D₂ and its related compounds.

EXPERIMENTAL

1. Materials

Vitamin D₂ and Its Related Compounds — The same materials as used in the previous reports (1, 2) were used.

Adsorbents — Kieselgel G and aluminium oxide G of Merck were used.

Developing Solvents — Three solvent systems made by mixing *n*-hexane and methyl ethyl ketone (10:2, 10:3 and 10:4) were used.

¹ 小林 正.

2. Procedure

A slurry was prepared by mixing Kieselgel G or aluminium oxide G and water according to the conventional method and was applied on clean glass plates to make a uniform thickness of $250\ \mu$. The plates were dried at 110° for one hour. Constant volumes of 2% solution of the samples in *n*-hexane were applied to the plates with a micropipet and the chromatograms were developed with the developing solvents. The solvent front was allowed to move 10 cm from the origin. After drying the chromatograms by standing in the air for a short time, the spots were detected by spraying with SbCl_3 reagent containing acetyl chloride.

RESULTS

TABLE 1
R_f Values of Thin Layer Chromatography

Adsorbent	Kieselgel G			Aluminium oxide G		
Developing solvent	<i>n</i> -Hexane-methyl ethyl ketone					
	10 : 2	10 : 3	10 : 4	10 : 2	10 : 3	10 : 4
Vitamin D ₂	0.35	0.42	0.57	0.40	0.48	0.60
5,6- <i>trans</i> Vitamin D ₂	0.48	0.50	0.63	0.54	0.68	0.75
Isocalciferol	0.49	0.52	0.62	0.50	0.65	0.75
Isotachysterol ₂	0.41	0.47	0.58	0.45	0.58	0.65
Tachysterol ₂	0.35	0.42	0.57	0.40	0.48	0.60
Lumisterol ₂	0.40	0.43	0.55	0.47	0.58	0.65
Ergosterol	0.29	0.37	0.45	0.35	0.40	0.50

The *R_f* values of the compounds in the three solvent systems are shown in Table 1. When the two adsorbents were compared, Kieselgel G was superior to aluminium oxide G because of its little tailing and little extension of the spots. On the other hand, the solvent system of *n*-hexane methyl ethyl ketone (10 : 2) was the most desirable among the three systems, because the best separation was obtained by the system. Fig. 1 shows the chromatogram obtained by the application of these best conditions. However, vitamin D₂ from tachysterol₂, 5,6-*trans* vitamin D₂ from isocalciferol, and isotachysterol₂ from lumisterol₂ were not separated even by the best condition of the chromatography as shown in Fig. 1.

FIG. 1 *Thin Layer Chromatograms of Vitamin D₂ and Its Related Compounds*

Adsorbent, Kieselgel G.

Developing solvent, *n*-hexane-ethyl ether (10 : 2).

Samples, (1) vitamin D₂; (2) 5,6-*trans* vitamin D₂; (3) isocalciferol; (4) isotachysterol₂; (5) tachysterol₂; (6) lumisterol₂; (7) ergosterol; (8) mixture of (1)–(7).

DISCUSSION

The separations of vitamin D₂ from tachysterol₂, 5,6-*trans* vitamin D₂ from isocalciferol, and isotachysterol₂ from lumisterol₂ were not obtained by the thin layer chromatography. These results, which were identical with the results obtained by the alumina column chromatography according to method I described in the previous report (2), showed that the method for the identification of vitamin D₂ isomers was not established by the thin layer chromatography only.

There are some difficulties to determine the ultraviolet spectra or to apply gas chromatography after thin layer chromatography, because the application of large quantities of the samples to thin layer chromatography is generally difficult. Therefore, it might be concluded that the thin layer chromatography was unsuitable for the identification of vitamin D₂ isomers.

SUMMARY

Thin layer chromatography for the identification of vitamin D₂, 5,6-*trans* vitamin D₂, isocalciferol, isotachysterol₂, tachysterol₂, lumisterol₂ and ergosterol was investigated, and the following results were obtained.

1. When Kieselgel G as an adsorbent and *n*-hexane-methyl ethyl ketone (10:2) as a developing solvent were used, the most desirable separation was obtained.
2. However, the separation of vitamin D₂ from tachysterol₂, 5,6-*trans* vitamin D₂ from isocalciferol, and isotachysterol₂ from lumisterol₂ was not possible even under the most desirable condition.
3. These results were identical with those obtained by the alumina column chromatography according to method I described in the previous report (2).
4. There are some difficulties to apply other identification methods after thin layer chromatography, because the application of large quantities of the compounds to the chromatography is generally impossible. Based on these observations, it was concluded that thin layer chromatography might be unsuitable for the identification of vitamin D₂ isomers.

ACKNOWLEDGEMENT

The author wishes to thank Professor Dr. Chikataro Kawasaki, Faculty of Pharmaceutical Sciences of Osaka University, and Dr. Shuntaro Ogawa, previous director of the Branch, for their helpful directions, and also wishes to thank Dr. Morizo Ishidate, Dr. Kazutaka Yamaguchi, Dr. Yoshitomo Kochi and Dr. Kenshu Mochida for their continuous interest and encouragement. The author is indebted to Philips-Duphar Co. in the Netherlands and IDCC, the Japan agent of the company, for supplying the materials used in this study.

REFERENCES

1. Kobayashi, T., *J. Vitaminol.* **13**, 255 (1967).
2. Kobayashi, T., *J. Vitaminol.* **13**, 258 (1967).
3. Dávidek, J., and Blattna, J., *J. Chromat.* **7**, 204 (1962).
4. Katsui, G., Ishikawa, S., Shimizu, M., and Nishimoto, Y., *Vitamins* **28**, 41 (1963).
5. Heaysman, L. T., and Sawyer, E. R., *Analyst* **89**, 529 (1964).
6. Norman, A. W., and Delluca, H. F., *Anal. Chem.* **35**, 1247 (1963).
7. Planta, C. V., Schwieter, U., Chopard-dit-Jean, L., Rüegg, R., Kofler, M., and Isler, O., *Helv. Chim. Acta* **45**, 548 (1962).
8. Kobayashi, T., *Vitamins* **32**, 477 (1965).