Isolation, Identification, and Biological Activity of 25-Hydroxy-24-oxovitamin D_3: A New Metabolite of Vitamin D_3 Generated by in Vitro Incubations with Kidney Homogenates†

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ABSTRACT: A metabolite of 25-hydroxyvitamin D_3 has been isolated in pure form from incubation mixtures containing kidney homogenates of chicks given large doses of vitamin D_3. The isolation involved methanol–chloroform extraction and six steps of column chromatography. The metabolite was identified as 25-hydroxy-24-oxovitamin D_3 by means of ultraviolet absorption spectrometry, mass spectrometry, infrared spectrometry, nuclear magnetic resonance spectrometry, and specific chemical reactions. Use of a sensitive in situ technique revealed that 25-hydroxy-24-oxovitamin D_3 enhances intestinal calcium transport in rats approximately as effectively as 24,25-dihydroxyvitamin D_3 does. In contrast, 25-hydroxy-24-oxovitamin D_3 appeared to be less active than 24,25-dihydroxyvitamin D_3 in chicks 24 h after intravenous injection.

In 1970, Suda et al. (1970a) isolated a metabolite of 25-hydroxyvitamin D_3 [25(OH)D_3][1] from the plasma of hogs given large amounts of vitamin D. This metabolite, initially proposed to be 21,25-dihydroxyvitamin D_3 (Suda et al., 1970a), was subsequently identified as 24,25-dihydroxyvitamin D_3 [24,25$(OH)_2D_3$] (Holick et al., 1972). It is produced in the kidney (Holick et al., 1972), intestine (Kumar et al., 1978), and possibly cartilage (Garabedian et al., 1978) by a mixed function oxygenase. Its production is controlled by the hormonal form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] (Tanaka & DeLuca, 1974; Tanaka et al., 1975). 24,25$(OH)_2D_3$ has significant biological activity in rats (Boyle et al., 1973) and chicks (Henry et al., 1976), but it is less active in chicks than in rats, probably due to more rapid metabolism and excretion (Holick et al., 1976). Apparently, 1α-hydroxylation is required for its activity in stimulating intestinal calcium absorption and bone mineral mobilization in rats (Boyle et al., 1973). In addition, 24,25$(OH)_2D_3$ has been reported to have specific actions in the mineralization of bone (Ornoy et al., 1978), proteoglycan synthesis of cartilage (Corvol et al., 1978), suppression of parathyroid hormone secretion (Canterbury et al., 1978, 1980), and chicken egg hatchability (Henry & Norman, 1978).

In the course of investigating renal 25(OH)D_3-24-hydroxylase activity, we have found that kidney homogenates from chicks supplemented with vitamin D_3 metabolize in vitro 25(OH)[^{3}H]D_3 to three other radioactive metabolites (peaks A, C, and E) besides 24,25$(OH)_2$[^{3}H]D_3 (Takasaki et al., 1978). The enzymes responsible for the production of peaks A and C appeared to be induced by 1α,25$(OH)_2D_3$ (Suda et al., 1979). Production of peak A increased in parallel with the increase of the amount of 25(OH)D_3, while that of peak C was fairly constant irrespective of the increase of the substrate (Suda et al., 1979). Peak A was a slightly less polar metabolite than 25(OH)D_3, and it was eluted in the 25(OH)D_3 fraction on Sephadex LH-20 columns (Takasaki et al., 1978). Peak A has now been isolated in pure form from chicken kidney incubations with 25(OH)D_3 and identified as 25-hydroxy-24-oxovitamin D_3 [25(OH)24-oxo-D_3]. It is the purpose of this report to establish the structure and biological activity of this new renal metabolite of 25(OH)D_3. A preliminary report of this work has appeared (Takasaki et al., 1980).

Experimental Procedures

Radioactive determinations were carried out with a Packard Tri-Carb Model 3255 liquid scintillation counter. Samples with tritium were dried in minivials in a stream of air and dissolved in a toluene counting solution containing 2 g of 2,5-diphenyloxazole in 1 L of toluene.

The ultraviolet spectrum was recorded in an ethanol solution with a Union Giken Model SM-401 spectrophotometer. Mass spectra were obtained by using a JEOL JMS-D300 mass spectrometer. All spectra were run at 70 eV with a source temperature programmed in the range of 50–300 °C at 100 °C/min. The infrared spectrum was obtained from a KBr tablet with a Jasco A-302 infrared spectrometer. The proton nuclear magnetic resonance spectrum was taken in $CDCl_3$ on a Varian XL-100 spectrometer, and the chemical shift values are reported in parts per million downfield from internal Me_4Si.

Vitamin D Compounds. Crystalline vitamin D_3 and 25-(OH)D_3 were purchased from Wako Chemical Co., Tokyo, Japan, and Phillips Duphar Co., Amsterdam, The Netherlands, respectively. Crystalline 24*R*,25$(OH)_2D_3$ was kindly donated by Dr. M. R. Uskokovič, Hoffmann-La Roche Inc., Nutley, NJ. 25(OH)[26,27-$^3H_2$]D_3 (9.0 Ci/mmol) was purchased from Radiochemical Centre, Amersham, England.

Isolation of the Metabolite. A total of 1350 1-day-old White Leghorn cockerel chicks were maintained for 10 days on a vitamin D deficient diet containing 1.1% calcium and 0.6% phosphorus. The animals were orally dosed with 650 nmol of vitamin D_3 48 h prior to sacrifice. The kidneys were quickly removed, rinsed, minced with a garlic press, and homogenized in 4 volumes of 0.2 M sucrose containing 15 mM Tris-HCl (pH 7.4), 2 mM $MgCl_2$, and 5 mM sodium succinate (Takasaki et al., 1978). A total of 4.6 L of the homogenate

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[1] Abbreviations used: 25(OH)D_3, 25-hydroxyvitamin D_3; 1α,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; 24,25$(OH)_2D_3$, 24,25-dihydroxyvitamin D_3; 25(OH)24-oxo-D_3, 25-hydroxy-24-oxovitamin D_3; LC, liquid chromatography; Me_4Si, tetramethylsilane; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol.

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Table I: Flow Sheet of Isolation of 25(OH)24-oxo-D_3 from Kidney Extracts

column no.	chromatography of extracts
1	2.2 × 50 cm Sephadex LH-20 (70 g); seven separate batches; 65:35 (v/v) $CHCl_3$–hexane; pool 25(OH)D_3 fraction
2	4 × 20 cm silica gel 60 (100 g); six separate batches; 2:1 (v/v) hexane–ethyl acetate; pool 25(OH)D_3 fraction
3	high-pressure LC on 7.9 mm × 30 cm, semipreparative μ-Porasil; four separate batches; 1% methanol in CH_2Cl_2; pool radioactive peak eluting before 25(OH)D_3
4	high-pressure LC on 4 mm × 30 cm, μ-Bondapak C_{18}; four separate batches; 15% H_2O in methanol; pool UV peak eluting before 25(OH)D_3
5 and 6	high-pressure LC on 4.6 mm × 15 cm, Zorbax-SIL; 3.5% 2-propanol in hexane; pool UV peak eluting before 25(OH)D_3

was obtained. Each 180-mL aliquot was incubated with 1 μCi (160 nmol) of 25(OH)[26,27-$^3H_2$]D_3 in a 2-L Erlenmeyer flask at 37 °C for 30 min. The reaction was terminated by the addition of 675 mL of methanol–chloroform (2:1 v/v). Extraction was performed as described by Gray et al. (1972).

A flow sheet of the procedures used in the isolation of the metabolite is presented in Table I. The concentrated extracts were divided into seven equal parts, and each was chromatographed on a 2.2 × 55 cm column packed with 70 g of Sephadex LH-20. The column was eluted with 65% chloroform in 1-hexane according to the method of Holick & DeLuca (1971). Seventy 10-mL fractions were collected, and 100 μL of each fraction was used for tritium determination. The 25(OH)D_3 (tubes 7–20) and the 24,25$(OH)_2D_3$ (tubes 28–50) fractions from each column run were separately pooled and concentrated. The pooled 25(OH)D_3 fraction was further chromatographed in six batches on a 4 × 20 cm column of silica gel 60 (Merck, 100 g) by using a solvent of 1-hexane–ethyl acetate (2:1 v/v). Sixty 20-mL fractions were collected. Each 25(OH)D_3 fraction monitored by radioactivity (tubes 30–45) was pooled and concentrated.

The 25(OH)D_3 fraction which, besides 25(OH)D_3, includes the metabolite referred to as peak A was divided into four parts, and each was applied to a high-pressure liquid chromatograph (LC), Waters HPLC Model 204, equipped with a semipreparative μ-Porasil column (7.9 mm × 30 cm). The solvent system was 1% v/v methanol in dichloromethane. Forty 1-mL fractions were collected at a flow rate of 2 mL/min, and the radioactivity of each fraction was counted (Figure 1). A radioactive peak migrating prior to 25(OH)D_3 was collected, divided into four parts, and chromatographed separately on a reverse-phase high-pressure LC using a μ-Bondapak C_{18} (4 mm × 30 cm) column with 15% v/v water in methanol. The major UV peak was collected and further purified on a straight-phase high-pressure LC (Zorbax-Sil column, 4.6 mm × 15 cm, Du Pont) with 3.5% v/v 2-propanol in 1-hexane. The major UV peak was pooled and rechromatographed on the same system. The sole UV peak obtained was used for structural identification and measurements of biological activity. A total of 35.2 μg (on the basis of UV absorption at 265 nm) or 37.0 μg (on the basis of specific activity of tritium) of the purified metabolite was obtained. We also isolated 138 μg of purified 24,25$(OH)_2D_3$ from 1.6 mg of 25(OH)D_3. The amount of the new metabolite isolated was 2.3% of the substrate added and 26.8% of the 24,25-$(OH)_2D_3$ produced.

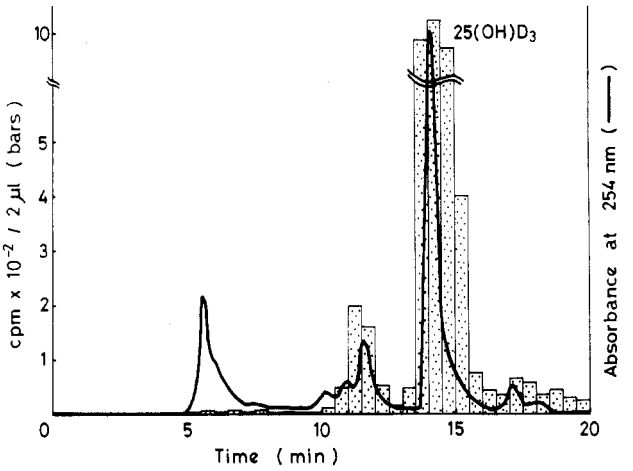

FIGURE 1: High-pressure LC profile (column no. 3) of the 25(OH)D_3 fraction collected from the silica gel 60 column.

Chemical Modification of the Metabolite. (*1*) *Trimethylsilylation.* The purified metabolite (1 μg) was dissolved in 10 μL of pyridine and reacted with 10 μL of *N,O*-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylsilyl chloride at 55 °C for 45 min. After evaporation of the solvent and excess of the reagents in vacuo, the residue was purified by high-pressure LC on a 0.39 × 30 cm μ-Porasil column eluted with 2.5% v/v ethyl acetate in 1-hexane. Two 265-nm absorbing peaks, the minor eluting between 3.2–3.8 mL and the major eluting between 4.5–5.2 mL, were collected and used for mass spectrometry.

(*2*) *$NaBH_4$ Reduction.* The metabolite (3 μg) was reduced with an excess of $NaBH_4$ in 5 μL of ethanol containing 0.1% of potassium hydroxide at 20 °C for 5 h. The mixture was diluted with ethyl acetate and washed with water. After evaporation of the solvent in vacuo, the residue was purified twice by high-pressure LC on a 0.39 × 30 cm μ-Porasil column eluted with 10% v/v 2-propanol in 1-hexane. The major 265-nm absorbing peak eluting between 10–12 mL was collected and used for mass spectrometry and further derivatization. The retention volume of the product was identical with that of authentic 24*R*,25$(OH)_2D_3$.

Periodate Oxidation of $NaBH_4$ Reduction Product. The $NaBH_4$ reduction product (350 ng) was dissolved in 10 μL of methanol and treated with 2 μL of a 5% aqueous solution of $NaIO_4$. After 4 h at 21 °C, the solvent was evaporated, and the residue was purified by high-pressure LC on a 0.39 × 30 cm μ-Porasil column eluted with 5% v/v 2-propanol in 1-hexane. The major 265-nm absorbing peak eluting between 6.3–8.0 mL was collected and subjected to mass spectrometry. The retention volume of the product was identical with that of 3β-hydroxy-9,10-secocholesta-5,7,10(19)-trien-24-al (Holick et al., 1972) obtained from authentic 24*R*,25$(OH)_2D_3$ by the same oxidation.

Measurement of Biological Activity. (*1*) *Animals.* Weanling male rats (Sprague–Dawley, body weight ~50 g) were raised for 5 weeks on a vitamin D deficient diet (Suda et al., 1970b) containing 0.2% calcium and 0.3% phosphorus. The animals were then placed on a low calcium (0.03%), vitamin D deficient diet for 3 days and injected intravenously with 975 pmol of 25(OH)24-oxo-D_3 or 24,25$(OH)_2D_3$ dissolved in 0.03 mL of ethanol, 24 h prior to the following assay. Control rats received ethanol only. For estimation of biological activity of the metabolite in chicks, 1-day-old White Leghorn cockerel chicks were fed a vitamin D deficient diet (Omdahl et al., 1971) containing 1% calcium and 0.45% phosphorus for 3 weeks. Twenty-four hours prior to the assay, the chicks were

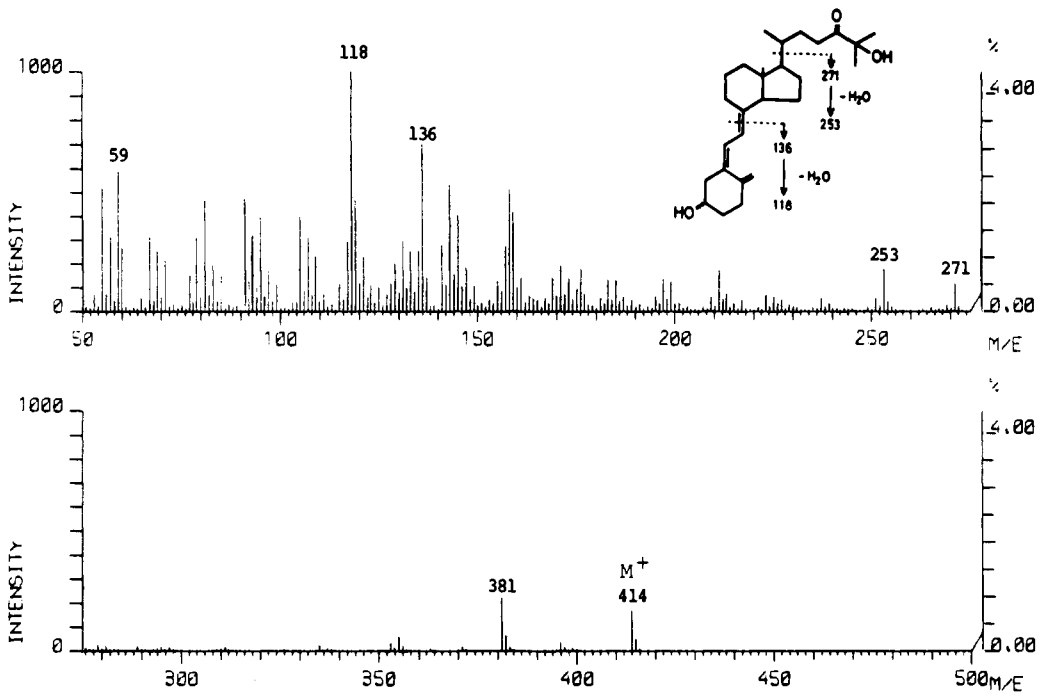

FIGURE 2: Mass spectrum of 25(OH)24-oxo-D_3.

dosed intravenously with 1.95 nmol of 25(OH)24-oxo-D_3, 25(OH)D_3, or 24,25-$(OH)_2D_3$.

(*2*) *Determination of Intestinal Calcium Absoption Activity.* Intestinal calcium absorption activity in both animals was estimated by using the sensitive duodenal loop in situ method originally used for chicks by Omdahl et al. (1971). The duodena of anesthetized rats were surgically exposed. After ligation of both sides of the 5-cm portions of the duodena, 0.2 mL of phosphate-free [^{45}Ca]calcium bicarbonate solution (120 mM NaCl, 4.9 mM KCl, 10 mM $CaCl_2$, 0.5 μCi/mL ^{45}Ca, and 9.2 mM $NaHCO_3$, pH adjusted to 7.0 with CO_2) was injected into the duodenal loop. The duodenal loop was replaced in the peritoneal cavity. Thirty minutes later the animals were killed by decapitation, and the blood was collected. The blood plasma was used for calcium determination.

The duodenum was rapidly excised, dried in a porcelain crucible at 100 °C for 12 h, and subsequently dry-ashed at 600 °C for 12 h. The ashed product was dissolved in 2 mL of 2 N HCl. Radioactivity was estimated by dissolving 100 μL of the aliquot in 10 mL of a dioxane counting solution (100 g of naphthalene and 5 g of 2,5-diphenyloxazole per L of dioxane) and by counting with a Packard liquid scintillation counter, Model 3255. Results were expressed as percent ^{45}Ca absorption and calculated by using the following formula: % $^{45}Ca_{abs} = 1 - {}^{45}Ca_R/{}^{45}Ca_A$, where $^{45}Ca_R$ is the amount of ^{45}Ca remaining in the duodenal loop following the in situ incubation and $^{45}Ca_A$ is the amount of ^{45}Ca initially added to the loop preparation.

(*3*) *Determination of Plasma Calcium Concentration.* Plasma calcium concentrations were measured by using an atomic absorption spectrometer (Hitachi, Model 170-50A). The rise in plasma calcium in rats fed a low-calcium diet reflects increased mobilization of bone mineral.

Results

Identification of Purified Metabolite as 25-Hydroxy-24-oxovitamin D_3. The ultraviolet spectrum of the purified metabolite showed a λ_{max} at 265 nm and a λ_{min} at 228 nm, demonstrating the presence of the 5,6-*cis*-triene chromophore characteristic of the D vitamins (Fieser & Fieser, 1959). The mass spectrum of the metabolite (Figure 2) showed molecular

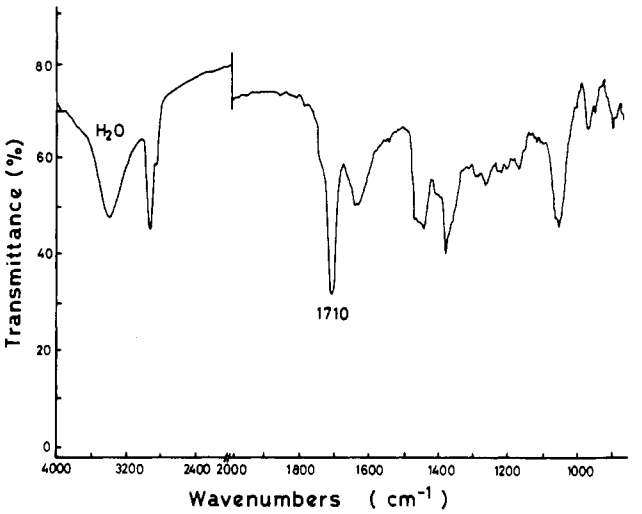

FIGURE 3: Infrared spectrum of 25(OH)24-oxo-D_3 (KBr disk). Transmittance (%) scale expansion is ×2 at 4000–2000 cm^{-1} and ×5 at 2000–900 cm^{-1}.

ion at *m/e* 414 suggesting the incorporation of an additional oxygen function into 25(OH)D_3 concomitant with the introduction of an unsaturation. The fragment ions at *m/e* 271, 253, 136, and 118, characteristic of the mass spectrum of the D vitamins (Blunt et al., 1968), indicate that the secosteroid nucleus of vitamin D has remained unchanged and that the metabolic alteration occurred on the side chain. The infrared spectrum (Figure 3) of the metabolite demonstrated the presence of a carbonyl group showing strong absorption at 1710 cm^{-1}. The 100-MHz proton nuclear magnetic resonance spectrum of the metabolite (Figure 4) showed the typical resonance of the olefinic protons of 5,6-*cis*-triene group of the D vitamins (Wing et al., 1975), an AB quartet centered at δ 6.17 (J = 11 Hz) for C-6 and C-7 protons and a pair of broad singlets at δ 4.84 and 5.08 for C-19 protons. The signals of the C-3 proton, δ 3.95 multiplet, and the 18-methyl group, δ 0.54 singlet, also appeared in the normal region of vitamin D. The sharp six-proton singlet at δ 1.38 which was assigned to the 26- and 27-methyl groups shifted 0.18 ppm downfield compared with that of the starting 25(OH)D_3, suggesting the

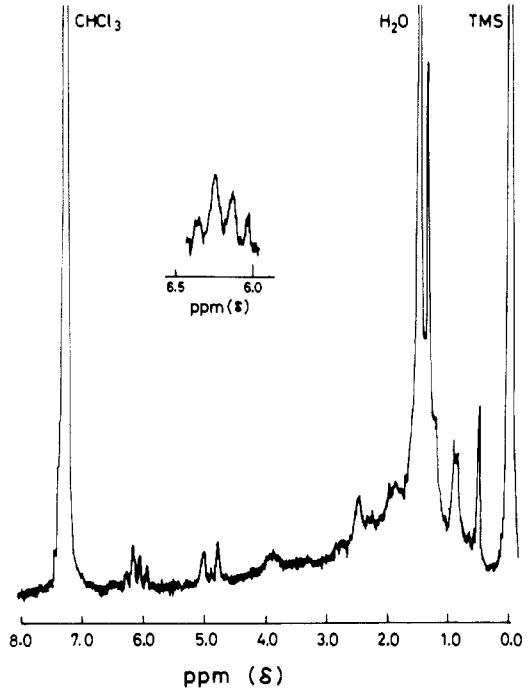

FIGURE 4: Proton nuclear magnetic resonance spectrum of 25-(OH)24-oxo-D_3 ($CDCl_3$). Insert shows the δ 6.0–6.5 region.

presence of a carbonyl functionality at the 24 position. This, together with the spectral data described above, suggested the structure of the metabolite to be 25-hydroxy-24-oxovitamin D_3 [25(OH)24-oxo-D_3].

Chemical transformations of the metabolite provided further supporting evidence for the assigned structure. Trimethylsilylation of the metabolite produced two silylated compounds. On the basis of the mass spectra, the major and more polar one was assigned to 3,25-bis(trimethylsilyl) ether and the minor and less polar one to 3,25-bis(trimethylsilyl) 24-enol trimethylsilyl ether. The diagnostic ions and the assignments are shown in Figure 5. The presence of the fragment ion at *m/e* 131 in both spectra demonstrated the $C_{26}(H_3)$–C_{25}(O-H)–$C_{27}(H_3)$ portion of the original 25(OH)D_3 was intact in the metabolite. Formation of enol trimethylsilyl ether on trimethylsilylation of ketone derivatives under similar reaction conditions has been known (Poole, 1978), and the result suggested that two hydroxyl groups as well as an enolizable ketone group were present in the metabolite.

Table II: Response of Intestinal Calcium Transport and Bone Mineral Mobilization Activities to 25(OH)24-oxo-D_3 and 24,25$(OH)_2D_3$ in Rats[a]

compound	intestinal calcium transport, % ^{45}Ca absorption	bone mobilization, serum Ca (mg %)
vehicle (control)	42.2 ± 2.5 (5)	5.9 ± 0.2 (6)
25(OH)24-oxo-D_3	65.8 ± 2.1 (5)[b]	6.6 ± 0.2 (5)[c]
24,25$(OH)_2D_3$	65.9 ± 2.4 (6)[b]	6.7 ± 0.3 (6)[c]

[a] The values are means ± SE. [b] Significantly different from control, $p < 0.001$. [c] Significantly different from control, $p < 0.05$.

$NaBH_4$ reduction of the metabolite produced a trihydroxy compound whose structure was determined to be 24ξ,25-$(OH)_2D_3$ on the basis of the mass spectrum and the chromatographic behavior. The mass spectral fragmentation pattern of the compound (Figure 6a) was nearly identical with the authentic 24*R*,25$(OH)_2D_3$ (Holick et al., 1972), and the retention volume of the compound on high-pressure LC was identical with that of the latter compound. Periodate oxidation of the reduction product confirmed the assigned structure. The oxidation afforded a cleavage product whose structure was determined to be 3β-hydroxy-9,10-secocholesta-5,7,10(19)-trien-24-al on the basis of the mass spectrum (Figure 6b) and chromatographic behavior compared with the authentic compound obtained from 24*R*,25$(OH)_2D_3$ by similar oxidation (Holick et al., 1972).

Biological Activity of Metabolite. Intestinal calcium transport response to 25(OH)24-oxo-D_3 was tested by the sensitive in situ method. At 24 h after intravenous administration of 975 pmol of the metabolites, 25(OH)24-oxo-D_3 was as active as 24,25$(OH)_2D_3$ in stimulating intestinal calcium transport activity in rats (Table II). 25(OH)24-oxo-D_3 also

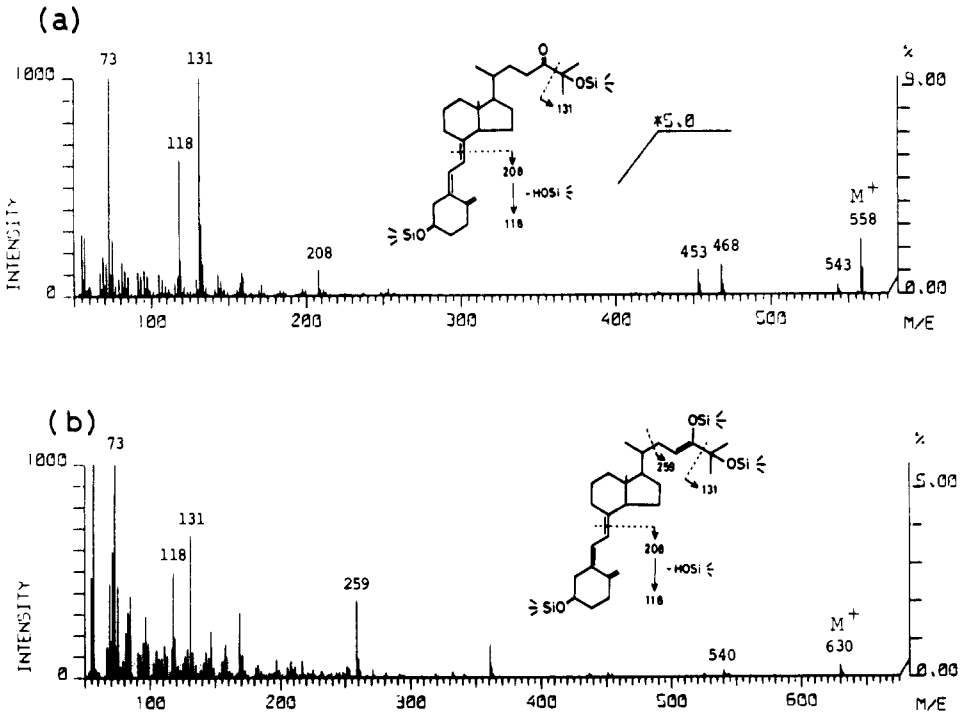

FIGURE 5: (a) Mass spectrum of the $(Me_3Si)_2$ derivative of 25(OH)24-oxo-D_3; (b) mass spectrum of the $(Me_3Si)_3$ derivative of 25(OH)24-oxo-D_3.

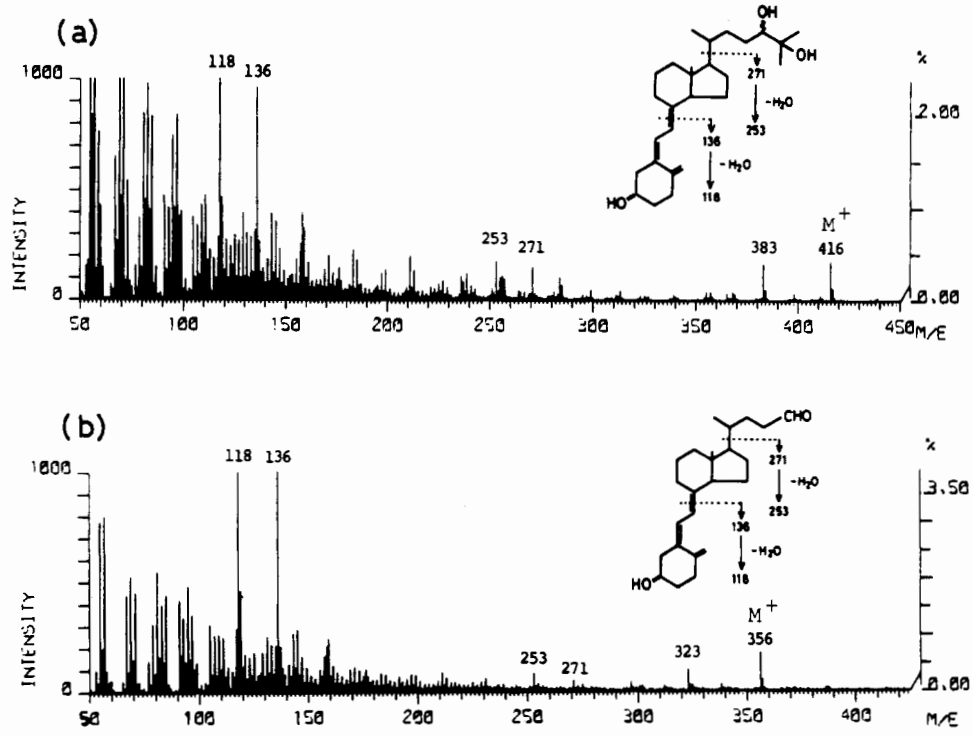

FIGURE 6: (a) Mass spectrum of 24ξ,25(OH)$_2$D$_3$ obtained from metabolite by $NaBH_4$ reduction; (b) mass spectrum of 3β-hydroxy-9,10-secocholesta-5,7,10(19)-trien-24-al obtained from 24ξ,25(OH)$_2$D$_3$ by $NaIO_4$ oxidation.

Table III: Response of Intestinal Calcium Absorption and Plasma Calcium Concentration to 25(OH)24-oxo-D_3, 25(OH)D_3 and 24,25(OH)$_2D_3$ in Chicks[a]

compound	intestinal calcium transport, % ^{45}Ca absorption	plasma calcium (mg/100 mL)
vehicle (control)	20.0 ± 1.3 (5)	8.9 ± 0.9 (5)
25(OH)24-oxo-D_3	28.5 ± 2.7 (3)[b]	9.3 ± 0.5 (3)
25(OH)D_3	68.9 ± 2.5 (4)[c]	10.5 ± 0.8 (4)
24,25(OH)$_2D_3$	47.8 ± 7.0 (4)[d]	10.0 ± 0.6 (4)

[a] The values are means ± SE. [b] Significantly different from control, $p < 0.02$. [c] Significantly different from control, $p < 0.001$. [d] Significantly different from control, $p < 0.01$.

appeared to have similar activity in enhancing bone mineral mobilization in rats. In contrast, 25(OH)24-oxo-D_3 seemed to be less effective than 24,25(OH)$_2D_3$ and 25(OH)D_3 in stimulating intestinal calcium transport activity in chicks. At 24 h after intravenous administration of 1.95 nmol of the metabolites, 25(OH)24-oxo-D_3 was only one-third as active as 24,25(OH)$_2D_3$ in chicks (Table III).

Discussion

The present report conclusively establishes the structure of peak A to be 25(OH)24-oxo-D_3. The identified metabolite appears to be synthesized from 25(OH)D_3 by way of 24,25-(OH)$_2D_3$ in the kidney of chicks supplemented with vitamin D_3. First, when kidney homogenates of chicks supplemented with vitamin D_3 were incubated with either 25(OH)[^{3}H]D_3 or 24,25(OH)$_2$[^{3}H]D_3, 25(OH)24-oxo-[^{3}H]D_3 (peak A) was similarly produced (Takasaki et al., 1978). Second, the homogenates did not produce 25(OH)[^{3}H]D_3 from 24,25-(OH)$_2$[^{3}H]D_3 (Takasaki et al., 1978). Third, when the homogenates were preheated at 100 °C for 5 min, peak A was not produced from 24,25(OH)$_2$[^{3}H]D_3 (Takasaki et al., 1978), indicating that 24,25(OH)$_2D_3$ is oxidized enzymatically to produce 25(OH)24-oxo-D_3. Fourth, kidney homogenates from vitamin D deficient chicks failed to produced peak A from 24,25(OH)$_2$[^{3}H]D_3 (Takasaki et al., 1978). Finally, we detected a radioactive peak with chromatographic behaviors similar to 25(OH)24-oxo-[^{3}H]D_3 in blood plasma of chicks given 24,25(OH)$_2$[^{3}H]D_3 (unpublished results). Thus, the possibility that the oxidation of the C-24 hydroxyl group of 24,25(OH)$_2D_3$ might have occurred during extraction and isolation procedures is excluded.

25(OH)24-oxo-D_3 was biologically active with approximately the same activity as 24,25(OH)$_2D_3$ in the rat. In contrast, 25(OH)24-oxo-D_3 appeared to be less active than 24,25(OH)$_2D_3$ in the chick 24 h after intravenous administration of the metabolites, though dose–response and time course studies are required for quantitation of the biological activity. It is well known that in the chick vitamin D_2 has only one-tenth the biological activity of vitamin D_3 (Chen & Bosmann, 1964); the only structural difference is the presence of a 22,23 double bond and a 24(*R*)-methyl group. It has also been reported that birds discriminate against vitamin D_2 by rapidly metabolizing and excreting its metabolites via the bile into the feces (Imrie et al., 1967). Norman et al. (1979) demonstrated that 24-nor-25(OH)D_3, a side-chain analogue of 25(OH)D_3, was inactive in the chick but exhibited a significant stimulation of both intestinal calcium transport and bone calcium mobilization in the rat. Thus, it appears that the chick vitamin D endocrine system is structurally more demanding than the rat system, particularly with regard to the side chain, and that the 24-hydroxylation of 25(OH)D_3 and the subsequent oxidation may be a route of inactivation of vitamin D_3. The biological activity of 25(OH)24-oxo-D_3 reported here is consistent with this assumption. It is assumed that 25(OH)24-oxo-D_3 has to be hydroxylated at the C-1 position before expressing its biological activity in the target tissues. However, whether or not the 24-ketone group of 25(OH)24-oxo-D_3 is reduced before or after 1α-hydroxylation must be taken into account in future investigations.

Acknowledgments

We are grateful to Dr. N. Horiuchi, E. Abe, N. Takahashi, T. Shinki, H. Horikawa, and T. Masumura for their assistance

in this project. We are also indebted to Dr. M. Sugahara, Research Laboratories of Amino Feed Co., for the use of facilities.

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