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## Note

# Gas chromatographic–mass spectrometric properties of boronate esters of 24*R*,25-dihydroxycholecalciferol

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The vitamin $D_3$ metabolite, 24*R*,25-dihydroxycholecalciferol (24,25-$(OH)_2$-$D_3$) has been shown to play a role in the control of formation of 1α,25-dihydroxycholecalciferol (1α,25-$(OH)_2$-$D_3$), a potent hormone[1]. 24,25-$(OH)_2$-$D_3$ is one of the more abundant metabolites, and some clinical interest attaches to its detection and measurement. Protein-binding measurement techniques are very sensitive but lack specificity. Methods utilizing high-performance liquid chromatography (HPLC) are sensitive enough to measure this compound in blood but problems of specificity still exist[2]. Gas chromatography–mass spectrometry (GC–MS) would offer the best combination of specificity and provide a suitable control method for measurements carried out using the other, more simple techniques.

Mass spectrometry has been applied during the initial structure elucidation of 24,25-$(OH)_2$-$D_3$[3] and the GC–MS properties of this and related compounds as their trimethylsilyl (TMS) ethers have been described[4,5]. During an investigation of a range of other derivatives for cholecalciferol and related seco-steroids[6] it was observed that the formation of a boronate ester between the 24- and 25-hydroxyls gave a much-improved mass spectrum by stabilization of higher mass ions, thereby increasing its potential for detection and measurement by GC–MS, particularly in the selected ion monitoring mode. The use of boronate esters has already been described for steroids with neighbouring hydroxyls[7,8]. The present paper describes some GC–MS properties of the methyl and *n*-butyl boronates of 24,25-$(OH)_2$-$D_3$ TMS and *tert.*-butyldimethylsilyl (TBDMS) ethers.

## EXPERIMENTAL

### *Samples and reagents*

24*R*,25-Dihydroxycholecalciferol was a gift from Drs. A. Fürst and W. Meier of Hoffmann-La Roche, Basel, Switzerland. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and a powerful silyating mixture (TMS reagent) comprising BSTFA, trimethylsilylimidazole and trimethylchlorosilane (3:3:2, v/v/v) were supplied by Serva (Heidelberg, G.F.R.). Methylboronic acid, *n*-butylboronic acid and a reagent mixture (TBDMS reagent) containing *tert.*-butyldimethylsilyl chloride (1 mmole),

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imidazole (2.5 mmoles) and dimethylformamide (to 1.5 ml) were purchased from Applied Science Labs., (State College, Pa., U.S.A.). Analytical grade solvents were obtained from E. Merck (Darmstadt, G.F.R.).

*Derivatization*

*Boronic esters–TMS ethers.* Samples of 24,25-$(OH)_2$-$D_3$ (20 $\mu$g) were treated with 20 $\mu$l of a solution of the *n*-butyl- or methylboronic acid (1 $\mu$g/$\mu$l) in tetrahydrofuran and left for 30 min at room temperature. After removal of solvent in a stream of nitrogen, the residue was treated with BSTFA (30 $\mu$l) for 30 min at room temperature. Excess reagent was then removed under a stream of nitrogen and the residue dissolved in 100 $\mu$l of ethyl acetate for GC–MS analysis.

*Boronic esters–TBDMS ethers.* Boronic esters were prepared as above and then treated with 20 $\mu$l of TBDMS reagent. After 30 min at room temperature, the mixture was filtered through a small column of Sephadex LH-20 (Pharmacia, Uppsala, Sweden) as described by Kelly and Taylor[9], and the product dissolved in 100 $\mu$l of ethyl acetate.

*Tris-TMS-ethers.* 24,25-$(OH)_2$-$D_3$ (20 $\mu$g) was treated with 20 $\mu$l of TMS reagent and after 5 min at room temperature the reaction mixture was filtered through Sephadex LH-20, as above. Solvent was then removed under a stream of nitrogen and the derivative dissolved in 100 $\mu$l of ethyl acetate.

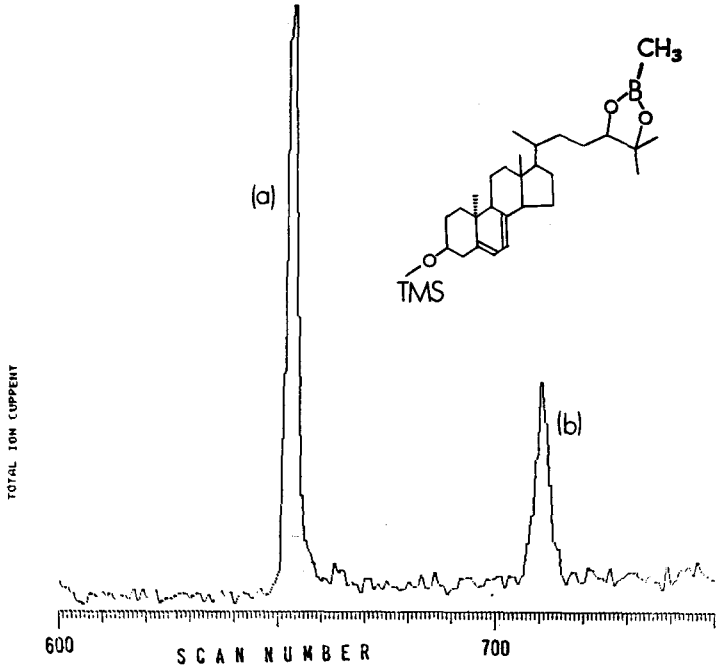


Fig. 1. Computer-reconstructed total ion current chromatogram of the 3-trimethhlsilyl ether-24,25-methylboronate ester derivative of 24*R*,25-dihydroxycholecalciferol showing the peaks a (scan number 653) and b (scan number 710) corresponding to both cyclized isomers (retention time relative to cholestane: 1.74 and 2.34, respectively). Chromatography carried out at 260° on a 50 m OV-17 capillary column; retention time of cholestane, 550 sec.

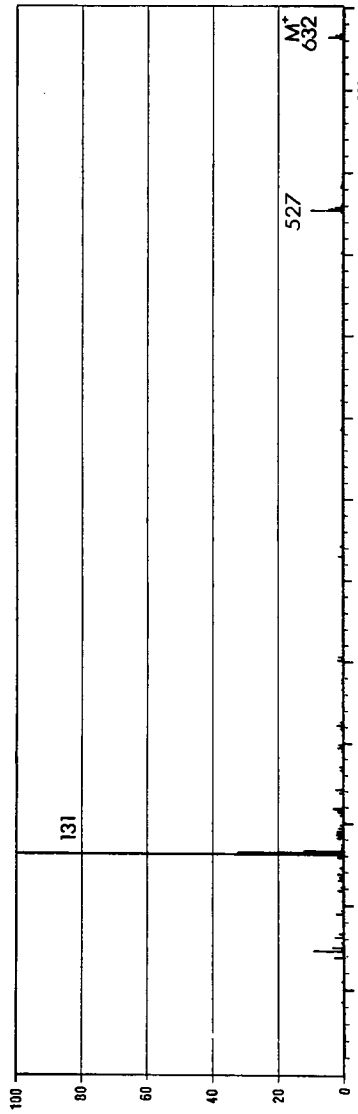


Fig. 2. Mass spectrum of 24*R*,25-dihydroxycholecalciferol-tris-trimethylsilyl ether (first and more abundant cyclized isomer) obtained on a 50 m OV-17 capillary column at 260°.

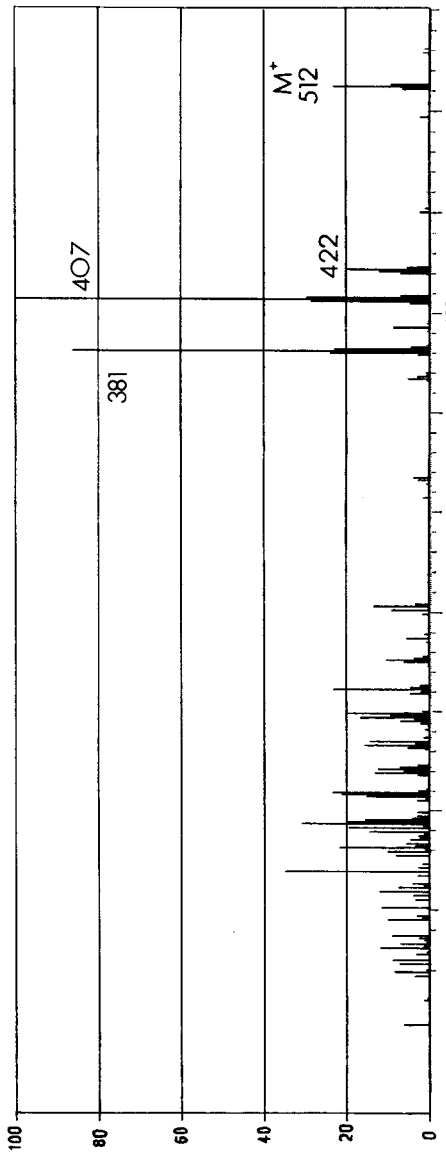


Fig. 3. Mass spectrum of the 3-trimethylsilyl ether-24,25-methylboronate ester derivative of 24*R*,25-dihydroxycholecalciferol (first and more polar cyclized isomers, retention time relative to cholestane 1.74) obtained on a 50 m OV-17 capillary column at 260°.

TABLE I

RETENTION INDICES ($I_{260}$) ON A 25-m OV-101 COLUMN AND IMPORTANT FRAGMENT IONS ($m/e$) FOUND FOR BOTH CYCLIZED ISOMERS OF 24*R*,25-DIHYDROXYCHOLECALCIFEROL AS TRIS-TRIMETHYLSILYL ETHER, 3-TRIMETHYLSILYL ETHER–24,25-METHYLBORONATE ESTER AND 3-TRIMETHYLSILYL ETHER–24,25-*n*-BUTYLBORONATE ESTER DERIVATIVES

| *Derivative* | $I_{260}$ | *Base peak* | *Important fragment ions (m/e) and abundance (in % base peak)* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| tris-TMS | | | | | | | | | | | | |
| Isomer I | 3434 | 131 | 632* (1) | 527§ (2) | 253 (1) | 158 (3) | 131 (100) | | | | | |
| Isomer II | 3579 | 131 | 632* (3) | 527§ (7) | 253 (1) | 131 (100) | | | | | | |
| Methylboronate–TMS | | | | | | | | | | | | |
| Isomer I | 3122 | 407 | 512* (45) | 487** (<1) | 422*** (24) | 407§ (100) | 381§§ (73) | 145 – | 143 (5) | 119 (3) | | |
| Isomer II | 3276 | 407 | 512* (99) | 422*** (56) | 407§ (100) | 381 (77) | 343§§ (15) | 253 (50) | 158 (82) | 145 (5) | 143 (43) | 119 (30) |
| *n*-Butylboronate–TMS | | | | | | | | | | | | |
| Isomer I | 3453 | 449 | 554* (48) | 464*** (25) | 449§ (100) | 423§§ (52) | 253 (7) | 211 (11) | 145 (10) | 143 (8) | 119 (6) | |
| Isomer II | 3575 | 554 | 554* (100) | 464*** (47) | 449§ (81) | 423§§ (40) | 253 (58) | 211 (29) | 158 (68) | 145 (35) | 143 (35) | 119 (19) |

* Molecular ion $M^+$.
** $M-15$.
*** $M-90$.
§ $M-(90+15)$.
§§ $M-131$.

*Gas chromatography–mass spectrometry*

An LKB 9000S system was used, equipped with open tubular capillary columns (LKB, Bromma, Sweden) coated with either OV-101 (25 m × 0.35 mm I.D.) or with OV-17 (50 m × 0.36 mm I.D.) "Falling needle"-type solid injection[10] was employed and the helium carrier gas flow-rate was *ca.* 2 ml/min, increased to 30 ml/min before the double-stage jet separator. Other conditions were: column temperature, 260°; separator temperature, 270°; ion source temperature, 290°; ionizing voltage, 22.5 eV; ionizing current, 60 μA; accelerating voltage, 3.5 kV. Amounts corresponding to 100–250 ng of stereol were injected and scanned spectra (4 sec scan time) recorded every 8 sec on magnetic disc, via an LKB 2130 data system.

Retention indices ($I_{260}$) of 24*R*,25-dihydroxycholecalciferol derivatives were calculated by comparison of their retention times at 260° with the times obtained for the hydrocarbons $C_{28}$ (614 sec), $C_{32}$ (1546 sec), $C_{34}$ (2542 sec), $C_{36}$ (4135 sec), $C_{38}$ (6718 sec) and $C_{40}$ (11,279 sec). The corresponding retention time of 5α-cholestane was 698 sec under these conditions (25 m OV-101 column).

TABLE II

RETENTION INDICES ($I_{260}$) ON A 25-m OV-101 COLUMN AND IMPORTANT FRAGMENT IONS (*m/e*) FOUND FOR BOTH CYCLIZED ISOMERS OF 24*R*,25-DIHYDROXYCHOLECALCIFEROL AS 3-TRIMETHYLSILYL ETHER–24,25-METHYLBORONATE ESTER AND 3-TRIMETHYLSILYL ETHER–24,25-*n*-BUTYLBORONATE ESTER DERIVATIVES

| *Derivative* | $I_{260}$ | *Base peak* | *Abundance of fragment ions m/e (in % base peak)* | | | | | | | | | *Others* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *M* | *M−15* | *M−57* | *M−59* | *M−132* | *M−133* | *M−134* | *M−(132+15)* | *M−173* | | | | |
| Methylboronate–TBDMS | | | 554 | 539 | 497 | 495 | 422 | 421 | 420 | 407 | 381 | 253 | 158 | 75 | |
| Isomer I | 3378 | 407 | 52 | 3 | 12 | 3 | 26 | 17 | 8 | 100 | 66 | 4 | 11 | 6 | |
| Isomer II | 3550 | 422 | 65 | 13 | 9 | 10 | 100 | 37 | 15 | 58 | 49 | 24 | 96 | — | |
| *n*-Butylboronate–TBDMS | | | 596 | 581 | 539 | 537 | 464 | 463 | 462 | 449 | 423 | 385 | 253 | 159 | 75 |
| Isomer I | 3686 | 449 | 58 | 4 | 10 | 6 | 26 | 26 | 15 | 100 | 59 | 9 | 14 | 16 | 24 |
| Isomer II | 3845 | 596 | 100 | 12 | 13 | 7 | 45 | 10 | — | 94 | 55 | 34 | 18 | 23 | 12 |

## RESULTS AND DISCUSSION

The computer-reconstructed total ion current chromatogram for the TMS–methylboronate derivative of 24,25-$(OH)_2$-$D_3$ is shown in Fig. 1. As expected for this seco-steroid, two peaks are obtained, corresponding to the major isomers (*pyro* and *isopyro*) formed by ring closure between C-9 and C-10 under the high-temperature conditions of the gas chromatograph[11].

The mass spectrum of the persilylated compound is shown in Fig. 2, and that of the TMS (3-hydroxy)–methyl boronate (24,25-dihydroxy) derivative in Fig. 3. In the former case, the *m/e* 131 ion accounts for nearly 100% of the ion current, and the fact that this ion lies in the lower mass region where contamination can be a problem makes it less suitable for either selected ion monitoring measurement or detection by mass chromatography. Both *pyro* and *isopyro* isomers of the tris-trimethylsilyl ether derivative and the trimethylsilyl ether (*n*-butylboronate) derivative have nearly identical retention times. If the boronate reaction is incomplete, the former derivative will be obtained to some extent, and its occurrence is easily seen by the presence of an abundant *m/e* 131 ion.

The mass spectrum of the methylboronate ester shows a molecular ion at *m/e* 512 having *ca.* 45% of the ion current (*pyro*) or 99% (*isopyro*), and a base peak *m/e* 407, $M-(90+15)$, in both cases. The second most abundant ion at *m/e* 381, $M-131$, includes loss of the methylboronate moiety (Table I).

Formation of *tert.*-butyldimethylsilyl ether–boronate derivatives yield mass spectra showing the same basic pathways of fragmentation as found in the corresponding trimethyl/boronate derivatives. In all boronic ester–TBDMS derivative mass spectra, the molecular ion has abundance greater than 50% of the base peak, and in the case of *isopyro*-butylboronate, the molecular ion is the base peak. Other abundant ions in all of the boronic ester–TBDMS derivatives are $M-132$, $M-(132+15)$ and $M-173$. The fragment $M-57$ is found to be rather small compared with those encountered in the spectra of other steroids[12]. The GC–MS characteristics of the various derivative combinations are given in Table II.

The results obtained show that boronate formation yields mass spectra of 24*R*,25-dihydroxycholecalciferol more suitable for detection and measurement by GC–MS and provide a striking example of the influence of choice of derivatives on the mass spectral fragmentation pattern.

## ACKNOWLEDGEMENTS

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