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Analysis of 2-Deoxybrassinosteroids by Gas Chromatography-Mass Spectrometry

SUGURU TAKATSUTO*,[a] and NOBUO IKEKAWA*,[b]

Department of Chemistry, Joetsu University of Education,[a] Joetsu, Niigata 943, Japan
and Department of Chemistry, Tokyo Institute of Technology,[b]
Ookayama, Meguro-ku, Tokyo 152, Japan

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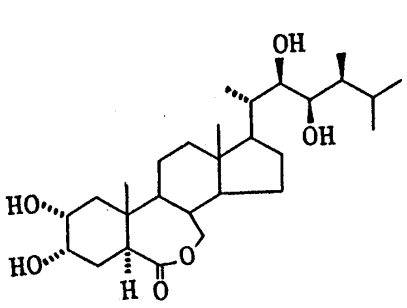
Analysis of four pairs of 3α,22,23- and 3β,22,23-trihydroxybrassinosteroids with modified side chains as their methaneboronate-trimethylsilyl (TMS) derivatives by gas chromatography-mass spectrometry (GC-MS) was carried out using a 1% OV-17 packed glass column and an electron impact ion source. In every case, the 3α-isomer had a much shorter retention time than the corresponding 3β-isomer. Both isomers showed almost the same fragment ions in their mass spectra, but their relative intensities were greatly different, particularly for the ions corresponding to the molecular ion minus 90 (TMSOH). These differences should be diagnostic for distinguishing the 3α- and 3β-isomers of 2-deoxybrassinosteroids from natural sources.

Keywords——brassinosteroid; 2-deoxybrassinosteroid; brassinolide; castasterone; typhasterol; teasterone; methaneboronate-trimethylsilyl derivative; GC-MS

Introduction

Since the discovery of a new plant growth hormone, brassinolide (**1**),[1] thirteen related bioactive steroids have been isolated and identified in a wide variety of higher plants.[2] In our previous papers,[3] we have reported that brassinolide (**1**), castasterone (**2**), and related compounds which have two vicinal diol functions at the side chain and the A-ring, could be analyzed as their bismethaneboronate derivatives by gas chromatography-mass spectrometry (GC-MS) and that this microanalytical technique is very effective for identifying brassinosteroids from plants sources, leading to the detection of these hormonal steroids in a wide range of higher plants.

During the course of our investigation of natural brassinosteroids in higher plants, we have isolated two new brassinolide-related 2-deoxysteroids, 2-deoxycastasterone (**3**)[4] and teasterone (**4**),[5] from the pollen of *Pinus thunbergii* Parl and the leaves of green tea (*Thea sinensis*), respectively. The 3α-steroid (**3**) has also been isolated by Schneider *et al.* from the cat-tail pollen (*Typha latifolia* L.) and named typhasterol.[6] These two 2-deoxysteroids were isolated together with castasterone (**2**) and brassinolide (**1**). Recently, Takahashi *et al.* have isolated teasterone (**4**), typhasterol (**3**), and castasterone (**2**) from the pollen of corn (*Zea mayz*).[7] These results suggest that the biosynthesis of brassinolide (**1**) may occur by the route **4**→ **3**→**2**→**1**, although another possible biosynthetic intermediate, 6-deoxocastasterone, was also isolated from the immature seeds of *Phaseolus vulgaris*, along with castasterone (**2**) and brassinolide (**1**).[8] Since the content of brassinosteroids in plants is generally much lower than that of other known plant hormones, a microanalytical method is absolutely necessary for their identification and characterization and also for biosynthetic studies. In this paper, we describe the GC-MS analysis of the 2-deoxybrassinosteroids (**3** and **4**) and related compounds as their methaneboronate-trimethylsilyl (TMS) derivatives. GC analysis of four stereoisomers of 20,22-dihydroxycholesterols and those of 22,23-dihydroxycholesterols as their methane-



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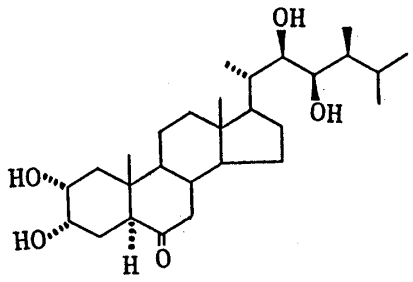

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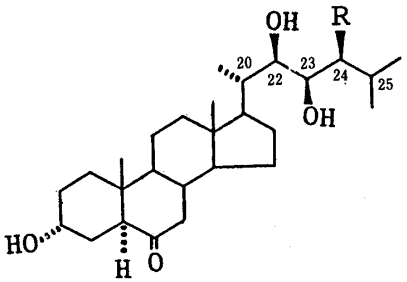
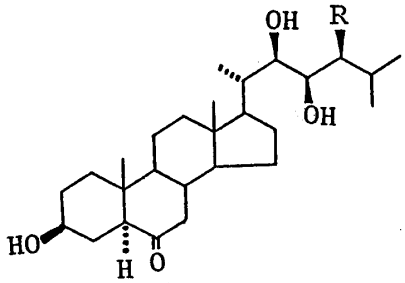
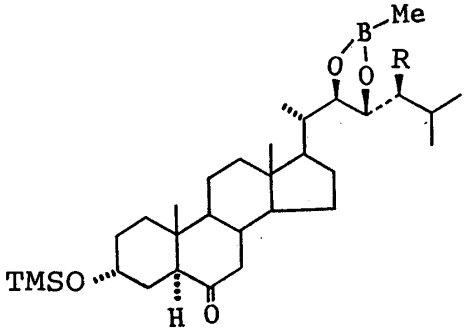


3: R=Me
5: R=Et
7: R=H
9: R=(*E*)=CHCH$_3$

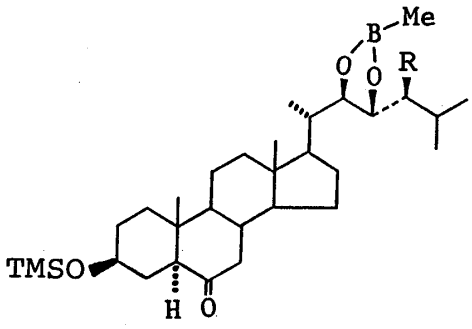


4: R=Me
6: R=Et
8: R=H
10: R=(*E*)=CHCH$_3$

Fig. 1. Structures of Brassinosteroids



3a: R=Me
5a: R=Et
7a: R=H
9a: R=(*E*)=CHCH$_3$



4a: R=Me
6a: R=Et
8a: R=H
10a: R=(*E*)=CHCH$_3$

Fig. 2. Structures of Methaneboronate-TMS Derivatives of 2-Deoxybrassinosteroids

boronate-TMS derivatives has already been reported in our previous paper.[3a)]

Results and Discussion

In order to establish the general trend of GC retention times and the mass fragmentation pattern of 2-deoxybrassinosteroids, four pairs of 2-deoxy-6-oxosteroids with modified side chains **3**—**10** were analyzed as their methaneboronate-TMS derivatives by GC-MS using a

TABLE I. GC Retention Times and Characteristic Fragment Ions (EI-MS) of Methaneboronate-TMS Derivatives of 2-Deoxybrassinosteroids

Compounds	t_R (min)	M^+ *m/z* (%)	M^+-15 *m/z* (%)	M^+-18 *m/z* (%)	M^+-29 *m/z* (%)	M^+-43 *m/z* (%)	M^+-90 *m/z* (%)	C_{20}–C_{22} cleavage *m/z* (%)
3a	6.2	544 (100)	529 (68)	526 (44)	515 (95)		454 (86)	155 (54)
4a	8.8	544 (36)	529 (84)		515 (100)		454 (10)	155 (20)
5a	7.0	558 (100)	543 (42)	540 (28)	529 (74)		468 (78)	169 (64)
6a	9.8	558 (42)	543 (74)		529 (100)		468 (14)	169 (82)
7a	5.3	530 (100)	515 (64)	512 (29)	501 (96)		440 (93)	141 (31)
8a	7.4	530 (38)	515 (92)		501 (100)		440 (10)	141 (13)
9a	7.1	556 (23)	541 (19)		527 (2)	513 (100)	466 (4)	167 (96)
10a	9.8	556 (14)	541 (18)		527 (8)	513 (80)	466 (6)	167 (100)

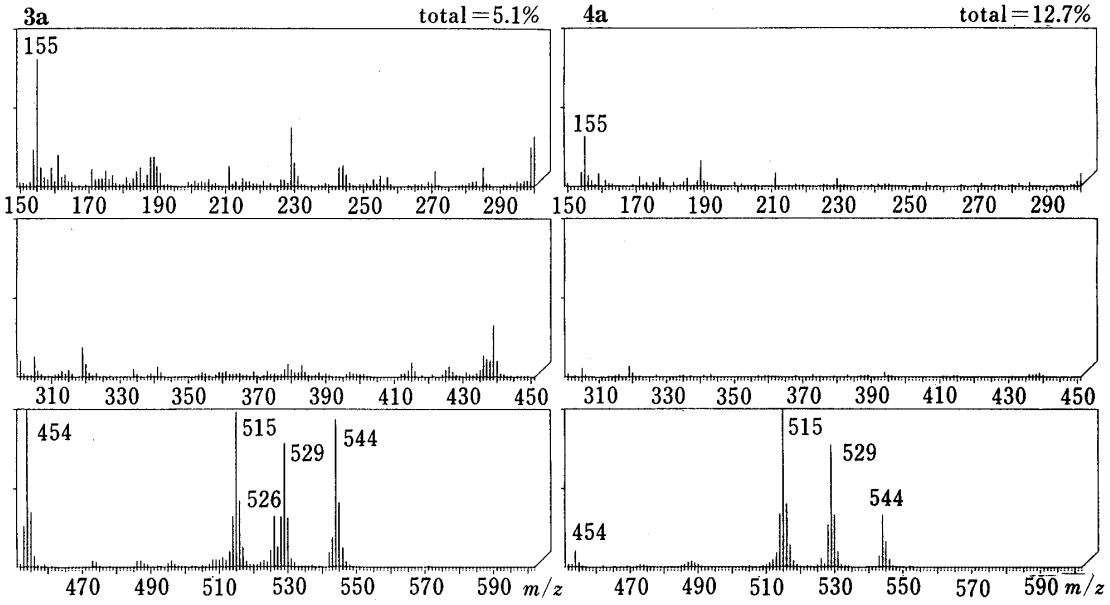


Fig. 3. Mass Spectra of Methaneboronate-TMS Derivatives **3a** and **4a** of Typhasterol (**3**) and Teasterone (**4**)

1% OV-17 packed column. In every case, the GC retention time of the 3α-derivative was much shorter than that of the corresponding 3β-derivative, as shown in Table I. Thus, GC retention time appears to be diagnostic for determination of the stereochemistry of the hydroxyl group at C-3 of 2-deoxybrassinosteroids.

In the electron impact (EI)-MS spectra (Fig. 3) of the methaneboronate-TMS derivatives **3a** and **4a** of typhasterol (**3**) and teasterone (**4**), the fragmentation patterns were almost identical except for the ion at *m/z* 526 (M^+-18) for the 3α-derivative **3a**, which was not observed for the 3β-derivative **4a**. The ion at *m/z* 526 may be formed by elimination of water from the enol form of the 6-oxo group and a hydrogen of the 3α-TMS group. The mass spectra of the derivatives **3a** and **4a** contain molecular ions at *m/z* 544 along with the ions at *m/z* 529 and 515, formed by elimination of one methyl and two methyls accompanied by H-transfer, respectively. The latter two ions were determined by measurement of high-resolution mass spectra. The spectra also contain prominent peaks at *m/z* 155 which result from the cleavage of the C_{20}–C_{22} bond. However, the relative intensities of the fragment ions were significantly different. The molecular ion at *m/z* 544 of the 3α-derivative **3a** was a base peak, while a base peak of the 3β-derivative **4a** was the ion at *m/z* 515, which represents the

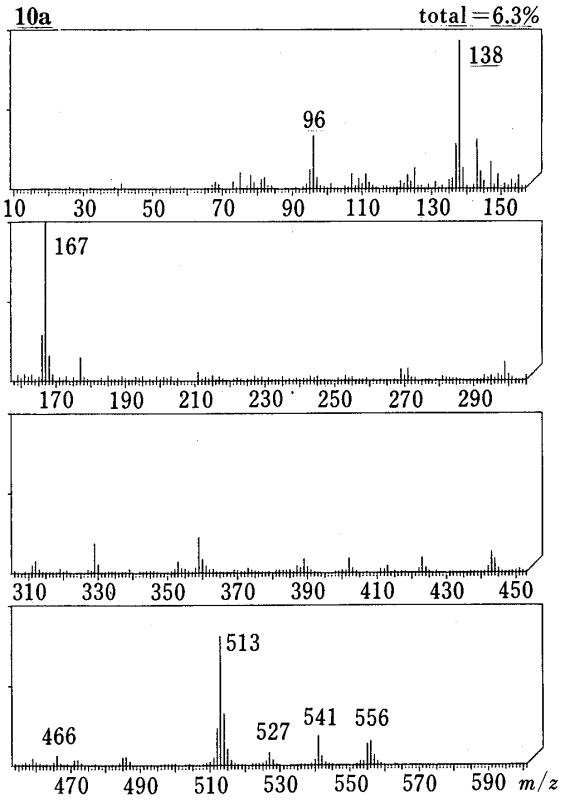


Fig. 4. Mass Spectrum of the Methaneboronate-TMS Derivative **10a** of 2-Deoxyhomodolichosterone (**10**)

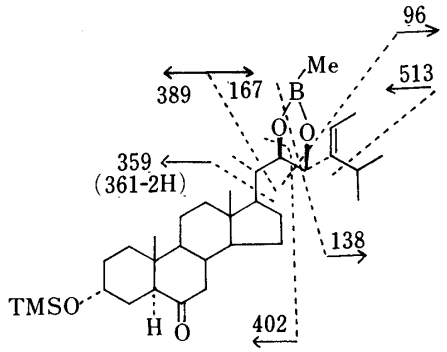


Fig. 5. MS-Fragmentation of the Methaneboronate-TMS Derivative **9a** of 2-Deoxyhomodolichosterone (**9**)

molecular ion minus 29. Another salient difference exists in the ion at *m/z* 454, which corresponds to elimination of TMSOH from the molecular ion at *m/z* 544; the intensity of this ion of **3a** is much higher than that of **4a**. The relative ease with which TMSOH is eliminated from the axially and equatorially orientated 3-trimethylsilyloxy steroids is reminiscent of the conformational considerations applicable to 3-hydroxysteroids.[9] These differences were also observed for other pairs of derivatives, **5a** *versus* **6a**, and **7a** *versus* **8a**. Therefore, these striking differences in fragmentation can be used for determination of the configuration of the 3-hydroxyl group of saturated 2-deoxybrassinosteroids.

In fact, based on these findings of relative GC retention time and fragmentation patterns of the mass spectra, we were able to determine the structure of teasterone (**4**) and typhasterol (**3**), which were obtained in very small amounts from green tea leaves.[5]

However, in the case of the unsaturated derivatives **9a** and **10a**, the differences seen in the mass spectra of the saturated derivatives were not observed, although their GC retention times were significantly different, as in the other cases. The mass spectra of a pair of isomers were identical in terms of fragmentation pattern and the relative intensities of the fragment ions; no salient difference was observed. This may be attributable to the facile formation of the resonance-stabilized allylic radical cation resulting from C-22, 23 fission, which is probably predominant over the enol radical cation of the 6-oxo group. The mass spectrum of the 3α-derivative **9a** contains a molecular ion at *m/z* 556 along with the ions at *m/z* 541, 527, and 466 which represent elimination of one methyl, two methyls accompanied by H-transfer, and TMSOH, respectively. In the unsaturated derivatives **9a** and **10a**, the ion at *m/z* 510 resulting from elimination of one molecule of water was not observed, while intense peaks at *m/z* 513 resulting from cleavage of the C_{24}–C_{25} bond were observed (Fig. 4). Another difference is the intense fragment ions at *m/z* 167, 138, and 96, which are derived from the

cleavage of the cyclic boronate moiety in the side chain part (Fig. 5). These cleavages were commonly observed for the bismethaneboronate derivatives of dolicholide, dolichosterone, homodolicholide, and homodolichosterone, which contain C_{24}–C_{28} unsaturation.[2b]

In conclusion, the 3α-isomers of methaneboronate-TMS derivatives of 2-deoxybrassinosteroids had much shorter retention times in GC than the corresponding 3β-isomers. Both isomers showed almost the same fragment ions, but for the saturated derivatives their relative intensities were greatly different, particularly the molecular ions and the ions corresponding to $M^+ - 90$, and these differences should be diagnostic for distinguishing the 3α- and 3β-isomers of 2-deoxybrassinosteroids from natural sources. In the case of the unsaturated derivatives no significant difference in their mass spectra was observed.

Experimental

Samples and Reagents——The following 2-deoxybrassinosteroids were synthesized in our laboratory; typhasterol (**3**),[10] teasterone (**4**),[11] their 28-homologues **5** and **6**,[12] their 28-nor analogues **7** and **8**,[11] and their 24-ethylidene analogues **9** and **10**.[11] Methaneboronic acid and trimethylsilylimidazole were obtained from Ventron Corporation and Tokyo Kasei Co., Ltd., respectively.

Derivatization——A solution of methaneboronic acid (10 μg) in dry pyridine (50 μl) was added to 10 μg of a 2-deoxybrassinosteroid. The mixture was heated at 70 °C for 30 min, then allowed to cool to room temperature, and trimethylsilylimidazole (10 μl) was added. The whole was left to stand at room temperature for 30 min. Several microliters were injected into the gas chromatograph-mass spectrometer.

GC-MS Analysis——A Shimadzu LKB 9000S gas chromatograph-mass spectrometer with an EI source was used. For GC-EI-MS, a glass column packed with 1% OV-17 on Chromosorb W (80—100 mesh) (2 mm i.d. × 1 m) was used at 280 °C; the carrier gas (helium) flow-rate was 50 ml/min; electron energy, 20 eV; electron current, 60 μA; acceleration high voltage, 3.5 kV; ion source temperature, 290 °C.

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