Pergamon

0031-9422(95)00363-O

Phytochemistry, Vol. 40, No. 4, pp. 1041–1043, 1995

0031-9422/95 $9.50 + 0.00

2-DEOXYBRASSINOLIDE—A NATURALLY OCCURRING BRASSINOSTEROID FROM *APIUM GRAVEOLENS*

JÜRGEN SCHMIDT, BRUNHILDE VOIGT and GÜNTER ADAM

Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle/S., Germany

(*Received* 25 *February* 1995)

Key Word Index—*Apium graveolens*; seeds; Umbelliferae; brassinosteroids; 2-deoxybrassinolide.

Abstract—A new brassinosteroid was detected in seeds of *Apium graveolens* and it was shown to be 2-deoxybrassinolide by gas chromatography–mass spectral analysis and comparison with suitable reference compounds.

INTRODUCTION

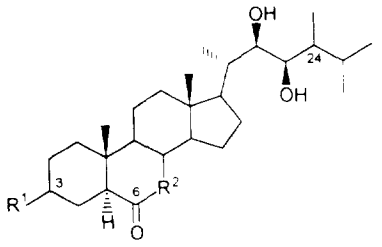
Brassinosteroids are a class of naturally occurring plant growth regulators with high biological activity occurring in a wide variety of plants from many families [1–3]. Up to now no occurrence of brassinosteroids has been described for plant species of the Umbelliferae family. Continuing our investigations of European cultivated plants we have investigated the seeds of *Apium graveolens* (celery) for brassinosteroids and have isolated 2-deoxybrassinolide (**1**). This paper describes the isolation and structure elucidation of this natural brassinosteroid.

RESULTS AND DISCUSSION

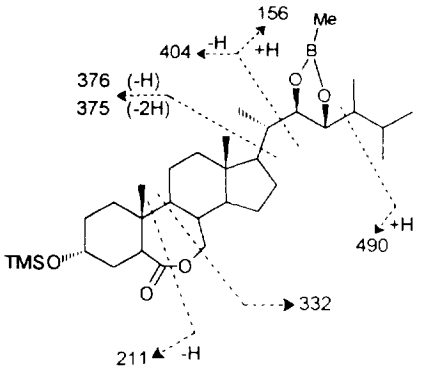
The powdered seed material was extracted with methanol, and the extracts were concentrated *in vacuo*. The residue was partitioned between chloroform and water. The chloroform extract was then partitioned between *n*-hexane and 80% methanol followed by repeated silica gel column chromatography of the concentrated 80% methanol extract using several methanol–chloroform gradient systems. The fraction eluted with 4% methanol showed bioactivity in the rice lamina inclination test. This fraction was concentrated and further purified on LH-20 Sephadex chromatography using methanol–chloroform (4 : 1) as eluent. The biological activity appeared in the eluates having 0.72–0.80 of the elution volume/total volume. The combined bioactive fractions from the Sephadex LH-20 chromatography were further purified by DEA ion exchange chromatography and finally by preparative reversed phase HPLC on an RP18 column using an acetonitrile–water system. The biologically active fractions with R_f values of 33–36 min (fraction A) and 49–53 min (fraction B) were analysed by GC-mass spectrometry (MS) after derivatization with methaneboronic acid followed by trimethylsilylation.

None of the known brassinosteroids could be identified in fraction A. There were hints for a new one with a castasterone type side chain and possibly two non-vicinal hydroxy groups at rings AB. However, a complete characterization was not possible. A new brassinosteroid (**1**, RR_t 2.06) was found in fraction B. The mass spectrum of the methaneboronate–trimethylsilyl derivative of **1** displayed a molecular ion at m/z 560. Both the $[M]^+$ ion and the key ions at m/z 545, 531 and 470 (losses of methyl, ethyl and trimethylsilanol, respectively) appeared with a mass shift of 16 amu compared with teasterone and typhasterol [4]. Ions at m/z 404 and 156 characterizing the side chain are complementary ions arising by cleavage of the bond C-20/C-22 (Scheme 1). The ion at m/z 332 also appearing in the mass spectrum of brassinolide is a key ion for lactone type brassinosteroids with hydroxyls at C-22 and C-23 as well as a methyl at C-24 [5]. Furthermore important key ions are m/z 376/375 (cleavage C-17/C-20), 211 (ring B cleavage), 195, 177 and 121 (m/z 211-TMSOH) (Scheme 1). The GC-MS data for the methaneboronate–trimethylsilyl derivative of compound **1** were compared with those of synthesized 2-deoxy-24-epibrassinolide (**2**) and 2-deoxy-3,24-diepibrassinolide (**3**), 24-epityphasterol (**5**) and 24-epiteasterone (**7**) (B. Voigt, unpublished) as well as typhasterol (**4**) and teasterone (**6**) (Table 1). The mass spectra of compounds **1** and **2** were identical, but quite different from that of **3**. However, in the GC, **1** eluted earlier than compounds **2** and **3**. The difference in the retention data for **1** and **2** (R_t and relative retention time RR_t) is typical for epimers with 24*S*- and 24*R*-configuration, respectively [5–7]. The same relationship was found for the 24-epimeric pairs, **4** and **5** and **6** and **7**, respectively. On the other hand, a comparison of the 3-epimeric pairs **2**/**3**, **4**/**6** as well as **5**/**7** with each other display a significantly larger retention time difference (Table 1). Therefore, compound **1** can be regarded as 2-deoxybrassinolide.

B-Homo-6a-oxa-lactone type brassinosteroids derived from **6** and **4** are hitherto known only as synthetic compounds [8]. Abe *et al.* [9] showed that the introduction of a lactone group in the ring B of 2-deoxybrassinosteroids has a promotive effect in the rice lamina inclina-



compound	R^1	R^2	configuration at C-24
1	α-OH	-O-CH_2-	24*S*
2	α-OH	-O-CH_2-	24*R*
3	β-OH	-O-CH_2-	24*R*
4	α-OH	-CH_2-	24*S*
5	α-OH	-CH_2-	24*R*
6	β-OH	-CH_2-	24*S*
7	β-OH	-CH_2-	24*R*



Scheme 1. Mass spectral fragmentation of the methaneboronate–trimethylsilyl derivatives of compounds **1** and **2**.

tion bioassay. Both **4** and **6** are intermediates in the biosynthetic pathway leading to castasterone and brassinolide [10]. The discovery of naturally occurring **1** indicates that, with regard to the substitution pattern at ring A, parallel biosynthetic pathways leading to oxalactone type brassinosteroids are possible.

EXPERIMENTAL

Plant material. The seeds of *Ap. graveolens* L. var. 'Apia' were obtained from 'Quedlinburger Saatgut GmbH', Quedlinburg, Germany.

Bioassay. The rice lamina inclination test was carried out using cv. 'Koshihikari' as described in ref. [11].

Extraction of brassinosteroids. The dried and powdered seeds (920 g) were extracted 3 × with MeOH. The combined MeOH extracts were evapd to dryness *in vacuo*. The residue was partitioned 3 × between H_2O and $CHCl_3$. The $CHCl_3$ phase was dried with Na_2SO_4, filtered off and evapd *in vacuo*. The residue (56.0 g) was partitioned between 80% MeOH (300 ml) and *n*-hexane (300 ml) and. The *n*-hexane phase was partitioned a 2 time with 80% MeOH, and the combined 80% MeOH frs were concd (36.2 g).

Purification of brassinosteroids. The residue resulting from the 80% MeOH fr. was chromatographed on a silica gel column (181 g). Elution was carried out with $CHCl_3$ (1 l), $CHCl_3$–MeOH (8:2, 1000 ml) and MeOH (1000 ml). The eluate with 20% MeOH (12.25 g) was chromatographed on a second silica gel column (60 g) using 150 ml of 2, 10 and 50% MeOH in $CHCl_3$. The 10% MeOH fraction (6.53 g) was then chromatographed on a silica gel column (32.5 g) and chromatographed stepwise with 10 frs (200 ml) of MeOH in $CHCl_3$ (0, 2, 3, 4, 5, 7, 10, 15, 30, 50%). The fr. eluted with 4% MeOH (167 mg) displayed biological activity and was evapd and further purified by LH-20 Sephadex CC (bed vol. 200 ml) with MeOH–$CHCl_3$ (4:1) as eluent. The eluates were collected in 5 ml frs. Frs 29–32 (elution vol./total column vol. 0.72–0.80) showing biological activity were combined and evapd. The residue (30.3 mg) was dissolved in MeOH and run on a DEA ion exchange cartridge (600 mg, Bond Elut). The residue resulting from the DEA CC (21.2 mg) was subjected to HPLC (Eurospher 80-C18, column 8 × 250 mm); flow rate, 2 ml min^{-1}, mobile phase, MeCN–H_2O (45% MeCN for 40 min, then raised to 80% MeCN within 5 min and held on 80% MeCN for 25 min, one run), 70 2 ml frs. The frs with activity (33–36 and 49–53) were pooled and concd and examined by GC-MS.

Table 1. GC-MS data for the methaneboronate–trimethylsilyl derivatives of compound **1** and the reference compounds **2–7**

Compound	RR_t*	Key ions in the EI mass spectra
1	2.06	560 $[M]^+$ (10), 545 (31), 531 (17), 490 (25), 470 (7), 404 (5), 376 (3), 375 (4), 332 (8), 287 (4), 211 (8), 195 (63), 177 (14), 156 (100), 121 (16), 85 (20)
2	2.13	560 $[M]^+$ (4), 545 (20), 531 (9), 490 (13), 470 (5), 404 (3), 376 (2), 375 (3), 332 (5), 287 (3), 211 (5), 195 (34), 177 (11), 156 (100), 121 (15), 85 (16)
3	2.59	560 $[M]^+$ (1), 545 (100), 531 (4), 489 (3), 391 (3), 375 (2), 269 (6), 213 (10), 169 (44), 156 (67), 121 (17), 107 (30), 93 (55), 85 (48)
4	1.61	544 $[M]^+$ (59), 529 (46), 526 (37), 515 (100), 454 (95), 439 (36), 436 (17), 319 (14), 305 (15), 299 (16), 229 (46), 211 (14), 155 (47), 121 (42), 85 (74)
5	1.66	544 $[M]^+$ (59), 529 (42), 526 (38), 515 (100), 454 (87), 439 (34), 436 (16), 319 (13), 305 (13), 299 (15), 229 (36), 211 (12), 155 (35), 121 (32), 85 (66)
6	1.78	544 $[M]^+$ (20), 529 (60), 515 (100), 454 (6), 319 (4), 305 (6), 229 (5), 211 (6), 155 (20), 121 (11), 85 (30)
7	1.85	544 $[M]^+$, (22), 529 (62), 515 (100), 454 (5), 319 (3), 305 (4), 229 (4), 211 (4), 155 (11), 121 (7), 85 (15)

*Relative retention time with respect to 5α-cholestane (R_t = 5.33 min).

GC-MS. MD-800 (Fisons Instruments); EI (70 eV); source temp. 200°; column DB-5MS (J&W, 15 m × 0.32 mm, 0.25 μm film thickness), inj. temp. 260°, column temp. programme: 170° for 1 min, then raised to 290° at 30° min^{-1} and held at this temp. for 20 min; interface temp. 300°, carrier gas He, flow rate 1 ml min^{-1}, splitless injection. The RR_t values were calculated with respect to 5α-cholestane (R_t = 5.33 min). The methaneboronation of the brassinosteroids was carried out with pyridine containing methaneboronic acid at 70° for 30 min [12]. After methaneboronation the samples were silylated with *N,O*-bis(trimethylsilyl)acetamide.

Acknowledgements—The authors wish to thank Mrs Helga Menzky for helpful technical assistance, Mrs Christine Kuhnt for the GC-MS measurements and Prof. Takao Yokota (Teikyo University, Utsunomiya, Japan) for gifts of authentic samples of teasterone and typhasterol, as well as the Deutsche Forschungsgemeinschaft (DFG) for financial support.

REFERENCES

1. Cutler H. G., Yokota, T. and Adam, G. (eds) (1991) *Brassinosteroids—Chemistry, Bioactivity, Application*. ACS Symposium Series 474, American Chemical Society, Washington, DC.
2. Sakurai, A. and Fujioka, S. (1993) *Plant Growth Regul.* **13**, 147.
3. Adam, G. and Petzold, U. (1994) *Naturwissenschaften* **81**, 210.
4. Abe, H., Morishita, T., Uchiyama, M., Takatsuto, S. and Ikekawa, N. (1984) *Agric. Biol. Chem.* **48**, 2171.
5. Ikekawa, N. and Takatsuto, S. (1984) *Mass Spectrosc. (Jpn)* **32**, 55.
6. Schmidt, J., Spengler, B., Yokota, T. and Adam, G. (1993) *Phytochemistry* **32**, 1614.
7. Schmidt, J., Spengler, B., Yokota, T., Nakayama, M., Takatsuto, S., Voigt, B. and Adam, G. (1995) *Phytochemistry* **38**, 1095.
8. Ando, T., Aburatani, M., Koseki, N., Asakawa, S., Mouri, T. and Abe, H. (1993) *Magn. Reson. Chem.* **31**, 94.
9. Abe, H., Asakawa, S., Ando, T., Mouri, T., Aburatani, M. and Takeuchi, T. (1992) *Biosci. Biotech. Biochem.* **56**, 1312.
10. Suzuki, H., Inoue, T., Fujioka, S., Takatsuto, S., Yanagisawa, T., Yokota, T., Murofushi, N. and Sakurai, A. (1994) *Biosci. Biotech. Biochem.* **58**, 1186.
11. Arima, M., Yokota, T. and Takahashi, N. (1984) *Phytochemistry* **23**, 1587.
12. Takatsuto, S., Ying, B., Morisaki, M. and Ikekawa, N. (1982) *J. Chromatogr.* **239**, 233.