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# SECASTERONE, THE FIRST NATURALLY OCCURRING 2,3-EPOXYBRASSINOSTEROID FROM *SECALE CEREALE*

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**Key Word Index**—*Secale cereale*; Gramineae; seeds; brassinosteroids; (22*R*,23*R*,24*S*)-22,23-dihydroxy-2β,3β-epoxy-24-methyl-5α-cholestan-6-one (secasterone).

**Abstract**—The new brassinosteroid secasterone, (22*R*,23*R*,24*S*)-22,23-dihydroxy-2β,3β-epoxy-24-methyl-5α-cholestan-6-one, isolated from seeds of *Secale cereale* (rye) was identified by gas chromatography-mass spectral analysis. Secasterone represents the first naturally occurring brassinosteroid with a 2,3-epoxy function. Furthermore, the known brassinosteroids castasterone, 28-homocastasterone, 28-norcastasterone (brassinone), 6-deoxocastasterone, typhasterol and teasterone were identified from seeds of the same plant material.

## INTRODUCTION

Brassinosteroids represent a class of naturally occurring plant growth regulators with high biological activity [1–3]. Their wide natural occurrence is also described for plants species of Gramineae family, *Oryza sativa* [4, 5] and *Zea mays* [6]. Recently, the first 3,6-diketobrassinosteroid (3-dehydroteasterone, 3-oxoteasterone), a possible intermediate in the biosynthetic pathway of brassinosteroids (teasterone → typhasterol), was isolated from lily anthers and leaves of *Distylium racemosum* [7] as well as from wheat (*Triticum aestivum* L.) grains [8]. This paper describes the isolation and structure elucidation of a new naturally occurring brassinosteroid possessing a 2,3-epoxy function as well as the identification of other brassinosteroids from seeds of *Secale cereale* L. (rye).

## RESULTS AND DISCUSSION

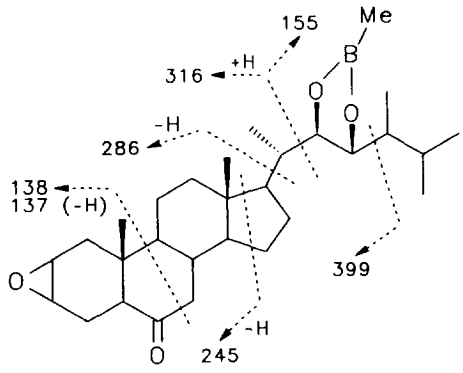
The seeds of *S. cereale* were extracted with methanol and the extracts were concentrated *in vacuo*. The aqueous residue was extracted with chloroform. The chloroform extract was partitioned between *n*-hexane and 80% methanol. The aqueous methanol fraction was subjected to a silica gel column eluted stepwise with increasing concentrations of methanol in chloroform. The fraction eluted with 5–15% methanol displayed bioactivity in the rice lamina inclination test. Further purification was carried out using LH-20 Sephadex chromatography with methanol–chloroform (4:1). The biological activity appeared in the eluates having 0.70–0.80 of the elution volume/total column volume. The bioactive fractions from the Sephadex LH-20 chromatography were combined and further purified by DEA chromatography followed by a C18-cartridge eluted with 75, 90 and 100% acetonitrile in water. The biologically active fraction (75% acetonitrile) was chromatographed by preparative HPLC on an Eurospher column using an acetonitrile–water system. The fractions with biological activity were combined and analysed by GC-mass spectrometry after methaneboronation or methaneboronation followed by silylation.

6-Deoxocastasterone, 28-norcastasterone (brassinone), castasterone, 28-homocastasterone, typhasterol and teasterone were identified by their GC-mass spectral data in comparison with authentic samples (Table 1). From the bioactive HPLC fractions 51–54 a hitherto unknown brassinosteroid could be isolated. In analogy to the recently described 3,6-diketo compound 3-dehydroteasterone (**5**) the mass spectrum of this compound obtained by GC-mass spectrometry after methaneboronation showed a molecular ion at *m/z* 470, but the GC retention data were quite different to **5**. Significant key ions ap-

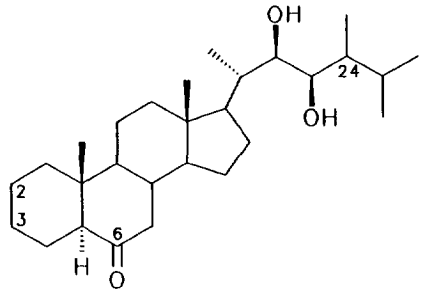
Table 1. GC data of the brassinosteroids from *Secale cereale*

| Compound | $RR_t^*$ | HPLC-fraction |
|---|---|---|
| 6-Deoxocastasterone | 0.818 | 25–26 |
| Brassinone | 0.912 | 25–26 |
| Secasterone | 0.935 | 51–54 |
| Castasterone | 1.000 | 29–30 |
| 28-Homocastasterone | 1.073 | 29–30 |
| Typhasterol | 0.927 | 53–54 |
| Teasterone | 1.048 | 53–54 |

*Relative retention time with respect to castasterone ($R_t$ = 11.82 min).




Scheme 1. Mass spectral fragmentation of the 2,3-epoxy-brassinosteroids **1**–**4**.




| Compound | Substitution at Ring A | Configuration at C-24 |
|---|---|---|
| **1** | 2α,3α-epoxy | 24*S* |
| **2** | 2β,3β-epoxy | 24*S* |
| **3** | 2α,3α-epoxy | 24*R* |
| **4** | 2β,3β-epoxy | 24*R* |
| **5** | 3-oxo | 24*S* |
| **6** | 3-oxo | 24*R* |

peared at *m/z* 454, 399, 316, 286, 245, 155 and 138/137 (Scheme 1). The base peak at *m/z* 155 gave a hint for a saturated side chain with two hydroxyls at C-22 and C-23 and the appearance of *m/z* 454 ($[M - O]^+$) for the presence of an epoxy function, respectively. In agreement with **5** both the molecular ion and other key ions showed a mass shift of 42 amu compared with castasterone. Therefore, this compound should also possess one oxygen function at ring A. To identify this compound unambigously we synthesized some reference compounds having a 2,3-epoxy function (Voigt, B., Yokota, T., Takatsuto, S. and Adam, G. unpublished data). The results of the GC-mass spectral investigation of the corresponding 24-epimeric 2α,3α-(**1**, **3**), 2β,3β-epoxy (**2**, **4**) as well as the 3,6-diketo derivatives (**5**, **6**) are given in Table 2. All six reference compounds could be separated by capillary GC. However, the 3,6-diketo derivatives **5** and **6** were quite different in their relative retention time ($RR_t$ with respect to castasterone) compared with the epoxy ones. The mass spectra of the 2α,3α-epoxy compounds **1** and **3** showed small differences in the abundance ratio of the ions *m/z* 454 and *m/z* 439 ($[M - O - Me]^+$) compared with the corresponding 2β,3β-compounds **2** and **4**. It should be pointed out that the fragmentation of compounds **1**–**4** is influenced by the ion source temperature. This is shown by an enhanced appearance of the ions at *m/z* 454 ($[M - O]^+$), *m/z* 439 ($[M - O - Me]^+$) and *m/z* 426 ($[M - O - CO]^+$), if the ion source temperature is raised to 250–300°.

However, the GC retention time ($RR_t = 0.935$ with respect to castasterone) and the mass spectrum of the isolated compound from *Secale cereale* (secasterone) are in agreement with the epoxy compound **2**. Therefore, secasterone could be regarded as (22*R*,23*R*,24*S*)-22,23-dihydroxy-2β, 3β-epoxy-24-methyl-5α-cholestan-6-one (**2**).

Secasterone represents the first naturally occurring brassinosteroid with a 2,3-epoxy function. It could be a metabolite or an intermediate within the metabolic sequence teasterone → typhasterol → castasterone, which needs further investigations.

Table 2. GC-mass spectral data of the synthesized 2,3-epoxy- and 3,6-dioxo-brassinosteroids **1**–**6**

| Compound | $RR_t$* | MS (key ions) |
|---|---|---|
| **1** | 0.920 | 470 ($M^+$, 80), 454 (29), 439 (31), 426 (12), 399 (2), 316 (17), 286 (13), 260 (9), 245 (29), 155 (100), 138 (29) |
| **2** | 0.941 | 470 ($M^+$, 87), 454 (16), 439 (11), 426 (5), 399 (9), 316 (26), 286 (8), 260 (10), 245 (24), 155 (100), 137 (6) |
| **3** | 0.950 | 470 ($M^+$, 67), 454 (28), 439 (31), 426 (11), 399 (10), 316 (23), 286 (11), 260 (16), 245 (33), 155 (100), 138 (22) |
| **4** | 0.972 | 470 ($M^+$, 100), 454 (17), 439 (6), 426 (4), 399 (12), 316 (31), 286 (12), 260 (8), 245 (17), 155 (93), 138 (13) |
| **5** | 0.991 | 470 ($M^+$, 49), 399 (8), 316 (22), 287 (12), 259 (8), 245 (23), 155 (100), 139 (9), 138 (7) |
| **6** | 1.020 | 470 ($M^+$, 42), 399 (8), 316 (14), 287 (11), 259 (7), 245 (19), 155 (100), 139 (10), 138 (11) |

*Relative retention time with respect to castasterone ($R_t$ = 10.98 min).

## EXPERIMENTAL

*Plant material.* The seeds of *Secale cereale* L var. 'Petka' were obtained from 'Samen Mauser GmbH', Quedlinburg, Germany.

*Bioassay.* The rice lamina inclination test was carried out using the cultivar 'Koshihikari' as described previously [9].

*Extraction of brassinosteroids.* The seeds of *S. cereale* (1175 g) were powdered and 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 $Na_2SO_4$. After filtration of the $Na_2SO_4$ the $CHCl_3$ was evapd. The residue (15.8 g) was partitioned between *n*-hexane (200 ml) and 80% MeOH (200 ml). The *n*-hexane phase was partitioned a second time with 80% MeOH and the combined 80% MeOH frs concd (2.6 g). The extract was dissolved in 5 ml $CHCl_3$–MeOH (4:1) and filtered. The residue was concd (2.4 g).

*Purification of the brassinosteroids.* The residue resulting from the 80% MeOH fr. was charged onto a column prepared by swelling of 11.5 g silica gel (Merck 60, 0.063–0.2 mm) in $CHCl_3$. Elution was carried out stepwise with 10 frs (120 ml) of MeOH in $CHCl_3$ (0, 2, 3, 4, 5, 7, 10, 15, 20, 50%). The frs eluted with 5–15% MeOH were biologically active. The 4 frs were combined and evapd. The residue (240 mg) was dissolved in a small amount of MeOH and further purified by a LH-20 Sephadex chromatography (bed vol. 500 ml) using MeOH–$CHCl_3$ (4:1) as eluent. The eluates were collected in 10 ml frs. Frs 35–40 (elution vol./total column vol. 0.70–0.80) were combined and evapd. The residue (78 mg) was dissolved in MeOH and run on a DEA ion exchange chromatography (1.6 g Bondesil 40 μm). The residue resulting from the DEA chromatography (46 mg) was chromatographed by a reversed phase cartridges (1 g C18, Bond Elut) and eluted with MeCN–$H_2O$ (75, 90 and 100% MeCN, each of this with 20 ml).

The fr. eluted with 75% MeCN was biologically active; after removal of the solvent the residue (38 mg) was subjected to HPLC (Eurospher 5-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 20 min, three runs) to provide 70 × 2 ml frs. The frs with activity were pooled and concd, and examined by GC-MS.

*GC-MS.* JEOL JMS-DX 505, EI (70 eV); source temp. 200°, column DB-1 (15 m × 0.25 mm, 0.25 μm film thickness), inj. temp. 260°, interface temp. 300°, carrier gas He, flow rate 1 ml $min^{-1}$ splitless injection; column temp. 170° for 1.5 min, then raised to 280° at a rate of 37 grd $min^{-1}$, from 280 to 298° at 1.5 grd $min^{-1}$. Methaneboronation of the brassinosteroids was carried out with pyridine containing methaneboronic acid at 70° for 30 min [10]. The HPLC frs 53–54 containing typhasterol and teasterone were silylated with BSA after methaneboronation.

*6-Deoxocastasterone.* EIMS *m/z* (rel. int.): 498 $[M]^+$ (100), 483 (39), 345 (12), 332 (11), 273 (88), 155 (49).

*28-Norcastasterone.* EIMS *m/z* (rel. int.): 498 $[M]^+$, (100), 358 (10), 329 (7), 287 (37), 141 (67).

*Secasterone.* EIMS *m/z* (rel. int.): 470 $[M]^+$, (82), 454 (12), 316 (49), 285 (23), 245 (22), 155 (100), 137 (9).

*Castasterone.* EIMS *m/z* (rel. int.): 512 $[M]^+$ (86), 441 (15), 399 (19), 358 (33), 287 (32), 155 (100).

*28-Homocastasterone.* EIMS *m/z* (rel. int.): 526 $[M]^+$ (32), 441 (13), 358 (27), 329 (6), 287 (16), 169 (100).

*Typhasterol.* EIMS *m/z* (rel. int.): 544 $[M]^+$ (100), 529 (45), 515 (82), 454 (16).

*Teasterone.* EIMS *m/z* (rel. int.): 544 $[M]^+$ (41), 529 (81), 515 (100), 454 (15).

*Synthesis of the reference compounds.* The synthesis of 3-dehydroteasterone (**5**) has already been published [7, 8]. The synthesis of the 2,3-epoxy compounds **1**–**4** as well as the 3,6-diketobrassinosteroid **6** (24-epi-3-dehydroteasterone) will be described elsewhere.

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