Pergamon

Tetrahedron Letters 40 (1999) 2359–2362

TETRAHEDRON LETTERS

Synthesis and Biological Activity of 6a-Carbabrassinolide: B-Ring Homologation of 6-Oxo-Steroid to 6-Oxo-7a-Homosteroid with Trimethylsilyldiazomethane–Boron Trifluoride Etherate

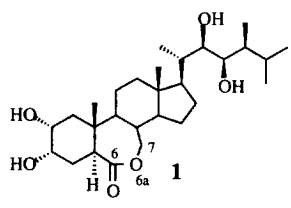
Hideharu Seto,* Shozo Fujioka, Hiroyuki Koshino, Hideki Hayasaka, Takeshi Shimizu, Shigeo Yoshida and Tsuyoshi Watanabe[a]

The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-0198, Japan
[a]Tama Biochemical Co., Ltd., 2-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 163-0704, Japan

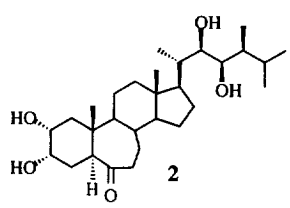
Received 16 December 1998; revised 11 January 1999; accepted 18 January 1999

Abstract: From castasterone (**10**), 6a-carbabrassinolide (**2**) was synthesized *via* a highly regioselective B-ring homologation with trimethylsilyldiazomethane and boron trifluoride etherate. A preliminary experiment using a simple 6-oxo-steroid (**3**) revealed that the actual products of this homologation reaction were α-trimethylsilyl ketones (**4**) and (**5**), which were converted to **6** (79%) and **7** (7.2%) by acid treatment. Biological activity of **2** in the rice lamina inclination test was *ca.* 1/20 compared with **1**, suggesting that 6a-oxygen on **1** is an important but not essential factor for the activity.

Brassinosteroids (BRs) are considered as a new class of phytohormones due to their physiological activities and ubiquitous distribution in the plant kingdom.[1] With increasing attention to the mode of action at physiological and molecular levels and the receptors, the structure-activity relationships of BRs have been widely studied and are now well documented on the basis of activity data of natural BRs and their synthetic analogs; among BRs, brassinolide (BL, **1**) exhibits the highest activity in various BR assays, which is generally ascribed to the lactone moiety of the B-ring, as well as the 2α,3α,(22*R*),(23*R*)-tetra-hydroxyl groups and A/B *trans* ring junction.[2,3] Although some structural modifications on the B-ring have been done so far,[3] it is still obscure whether the seven-membered lactone is really crucial for the high activity or not. We thought that replacement of 6a-oxygen on **1** to the carbon atom should be a good modification to probe the structural requirements of the B-ring for BR activity, but 6a-carbaBL (**2**) has not yet been prepared and assayed.[4] In this paper we report the preparation of **2** from castasterone (**10**), the immediate precursor of **1** in BR biosynthesis, *via* B-ring homologation with trimethylsilyldiazomethane ($TMSCHN_2$) in the presence of boron trifluoride etherate ($BF_3{\cdot}Et_2O$) and the result of the rice lamina inclination test on **2**, a typical bioassay employed for testing BR activity.[5] In this context, we also give the result of the homologation of a simple 6-oxo-steroid (**3**), which showed that the actual products of this



1

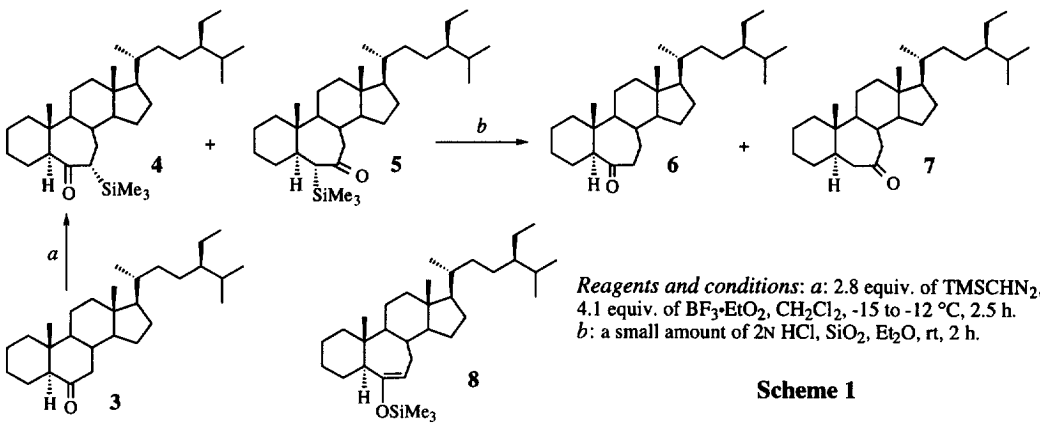


2

0040-4039/99/$ - see front matter
PII: S0040-4039(99)00186-0

reaction were α-trimethylsilyl ketones.

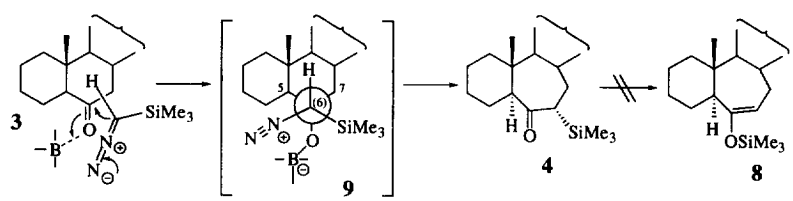
For the homologation of ketones, many reagents and conditions have been developed hitherto.[6] Among them, $TMSCHN_2$ developed by Shioiri *et al.* as an advanced substitute for diazomethane (CH_2N_2) seems to be suitable for our purpose, because a combination of this reagent and BF_3•Et_2O or organoaluminum Lewis acid allows a single-step homologation of ketones.[7] Since application of this reagent to 6-oxo-steroids has not yet been reported, it was first applied to a simple 6-oxo-steroid, 5α-stigmastan-6-one (**3**),[8] to examine the efficiency and the product selectivity. Upon treatment of **3** with 2.8 equiv. of $TMSCHN_2$ and 4.1 equiv. of BF_3•Et_2O in CH_2Cl_2 at –15 to –12 °C for 2 h followed by aqueous work-up, a mixture of homologated compounds was obtained, which was found by the ^1H NMR spectrum to mainly consist of 7α-trimethylsilyl-7a-homo-5α-stigmastan-6-one (**4**) and 7a-homo-5α-stigmastan-6-one (**6**).[9] For the complete desilylation, the mixture was then treated with a small amount of 2N HCl in the presence of silica gel in ether at rt for 2 h, affording **6** (mp 102-104 °C) in 79% isolated yield along with 7.2% of 7a-homo-5α-stigmastan-7-one (**7**) (mp 100-102 °C).



Reagents and conditions: *a*: 2.8 equiv. of $TMSCHN_2$, 4.1 equiv. of BF_3•EtO_2, CH_2Cl_2, -15 to -12 °C, 2.5 h. *b*: a small amount of 2N HCl, SiO_2, Et_2O, rt, 2 h.

Scheme 1

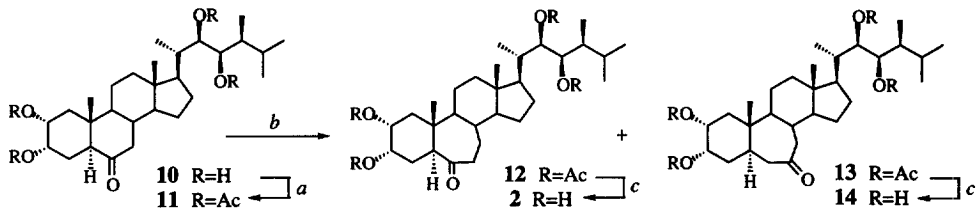
Identification of **4** is worthy of comment, because such α-trimethylsilyl ketone postulated as an intermediate in this homologation reaction[7a] has not been identified so far. The highly regio- and stereo-selective formation of **4** should reasonably explain the regio- and stereo-chemical course of this reaction and its successful single homologation. $TMSCHN_2$ attacks the BF_3-complexed carbonyl function of **3** preferentially from the axial site, *i.e.*, the hindered β-face,[10] with an orientation where the bulky trimethylsilyl group is located in the least-congested side and the hydrogen in the most-congested side, as illustrated in Scheme 2. A rigid staggered conformer of the adduct (**9**) satisfies the stereoelectronic requirements for the Tiffeneau-Demjanov type rearrangement of the C(6)-C(7) bond: the leaving diazonium group is oriented antiperiplanar to the C(6)-C(7) bond, while coperiplanar to both the C(5)-C(6) and C(6)-O bonds. Thus, **4** was formed selectively without the formation of epoxide, a possible by-product of this reaction.[7,11] The steric congestion around the carbonyl function of **4** and **5** being assumed as the precursor of **7** should inhibit further attack of $TMSCHN_2$, realizing the



Scheme 2

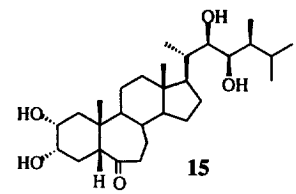
selective single-homologation. In this connection, trimethylsilyl enol ethers like **8** have been speculated on occasion to be derived from intermediary α-trimethylsilyl ketones and thus to be the actual products responsible for the selective single-homologation,[7] but no olefinic resonance due to enol ethers was observed at all in the ^1H NMR spectrum of the reaction mixture.

On the basis of the above preliminary experiment, 6a-carbaBL (**2**) was synthesized as follows (Scheme 3). Tetraacetate (**11**), derived from castasterone (**10**) [Ac_2O, pyridine, 4-dimethylaminopyridine, room temperature, 15 h, 98%, mp 209-212 °C: lit.,[12] mp 215-217 °C], was treated with $TMSCHN_2$ and BF_3•Et_2O in CH_2Cl_2 at −15 to −12 °C for 2.5 h, followed by acid treatment, affording 6-oxo compound (**12**) (mp 188-189 °C) and 7-oxo compound (**13**) (mp 231-234 °C decomp.) in 80 and 7.5% isolated yields, respectively. Compounds **12** and **13** were then subjected to deprotection reaction of the tetra-*O*-acetyl groups: on treatment with 5% potassium hydroxide in 90% aqueous methanol at refluxing temperature for 2 h, **12** furnished 6a-carbaBL (**2**) (mp 230-231 °C) in 96% yield along with a small amount of 5-*epi*-6a-carbaBL (**15**) (3.3%, an amorphous powder), while **13** gave the isomeric ketone (**14**) (mp 236–238 °C) quantitatively.



Reagents and conditions: *a*: Ac_2O, 4-DMAP, pyridine, rt, 15 h. *b*: i. 2.8 equiv. of $TMSCHN_2$, 4.3 equiv. of BF_3•EtO_2, CH_2Cl_2, -15 to -12 °C, 2.5 h. ii. a small amount of 2N HCl, SiO_2, Et_2O, rt, 2 h. *c*. 5% KOH-90% aq. MeOH, reflux, 2 h.

Scheme 3



Biological activity of 6a-carbaBL (**2**) in the rice lamina inclination test[5] was examined and compared with that of BL (**1**) (Table). The result shows that **2** exhibits *ca.* 1/20 activity of **1**, suggesting that 6a-oxygen on **1** is an important but not essential factor for the activity. The activity of the isomeric ketone (**14**) was also examined, but little activity was observed at tested dosages.

Compound	Dosages (ng)				
	0	1	10	100	1000
1	14±1	34±3	77±4	101±4	110±3
2	–	18±1	21±1	68±1	91±1
14	–	14±1	16±1	18±1	22±2

Table. Rice lamina inclination test (*Oryza sativa* cv. Tan-ginbozu) of **1**, **2** and **14**. Values are angles (°) between the lamina and sheath, representing the means of 30 replicates ± SE.

As described, $TMSCHN_2$–BF_3•Et_2O was found to be a good reagent for highly regioselective B-ring homologation of 6-oxo-steroid to 6-oxo-7a-homosteroid. It should provide an easy approach to B-ring homo analogs of biologically active steroids: to date, little has been known about how the B-ring homologation affected their biological activities. In a synthetic sense, the actual product of this homologation reaction, 7-trimethylsilyl-6-oxo-steroid, is interesting, because it sets the stage for diverse elaboration of the B-ring. As well as further structural modification of BRs starting from **2**, the optimization of the isolation procedure and study on reactivities of 7-trimethylsilyl-6-oxo-steroid are being undertaken.

Acknowledgment: This work was supported in part by Grant-in-Aid for Scientific Research (C) to H. Seto (No. 09672183) from the Ministry of Education, Science, Sports and Culture of Japan.

References and Notes

1. For a review, see Fujioka, S.; Sakurai, A. *Nat. Prod. Rep.* **1997**, *14*, 1-10.
2. Marquardt, V.; Adam, G. in *Chemistry of Plant Protection* Ebing, W. Ed.-in-Chief; Springer-Verlag: New York, 1991; vol. 7, pp. 103-139; Seto, H.; Fujioka, S.; Koshino, H.; Suenaga, T.; Yoshida, S.; Watanabe, T.; Takatsuto, S. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3355-3358; and references cited therein.
3. Okada, K.; Mori, K. *Agric. Biol. Chem.* **1983**, *47*, 89-95; Kishi, T.; Wada, K.; Ciuffreda, P.; Fiecchi, A.; Scala, A. *J. Org. Chem.* **1985**, *50*, 321-325; Anastasia, M.; Allevi, P.; Ciuffreda, P.; Fiecchi, A.; Scala, A. *J. Chem. Soc., Perkin Trans. 1* **1986**, 2117-2121; Anastasia, M.; Allevi, P.; Marumo, S.; Mori, K. *Agric. Biol. Chem.* **1986**, *50*, 1821-1830.
4. During the course of our work, a preceding paper on some novel brassinolide analogs including **2** appeared: Back, T. G; Baron, D. L.; Luo, W.; Nakajima, S. K.; Janzen, L.; Pharis, R. P. *Proceedings of the 24th Annual Meeting of the Plant Growth Regulation Society of America*, Atlanta, GA, 1997, pp. 107-110. However, the synthetic method and detailed biological activity of **2** can not be found out therein.
5. Fujioka, S.; Noguchi, T.; Takatsuto, S.; Yoshida, Y. *Phytochemistry* **1998**, *49*, 1841-1847.
6. Coveney, D. J. in *Comprehensive Organic Syntheses*, ed. Trost, B. M.; Fleming, I., Pergamon Press, Oxford, 1991, vol. 3, pp. 777-801; and references cited therein.
7. (a) Hashimoto, N.; Aoyama, T.; Shioiri, T. *Tetrahedron Lett.* **1980**, *21*, 4619-4622; Hashimoto, N.; Aoyama, T.; Shioiri, T. *Chem. Pharm. Bull.* **1982**, *30*, 119-124. (b) Maruoka, K.; Concepcion, A. B.; Yamamoto, H. *Synthesis* **1994**, 1283-1290; Mori, Y.; Yaegashi, K.; Furukawa, H. *J. Am. Chem. Soc.* **1997**, *119*, 4557-4558; Yang, S.; Hungerhoff, B.; Metz, P. *Tetrahedron Lett.* **1998**, *39*, 2097-2098.
8. Compound **3** was prepared from stigmastan-2,22-dien-6-one[13] by catalytic hydrogenation [H_2, 5% palladium on charcoal, AcOEt, rt, 1 atom, 2 h, 98%, mp 109 °C].
9. A careful work-up gave a mixture mainly containing *ca.* 9:1 of **4** and **6**: the reaction mixture was poured into ice-water and extracted with ether; the extract was washed with 2.5% $NaHCO_3$ and brine, and dried over Na_2SO_4. The NMR analysis of this mixture elucidated the structure of **4**, the stereochemistry of the C-7 being deduced by NOE experiment: ^{1}H NMR (600 MHz, C_6D_6): δ 0.04 (9H, s, -Si(CH_3)$_3$), 0.60 (3H, s, 18-H_3), 0.91 and 0.93 (each 3H, each d, *J*=6.8 Hz, 26- and 27-H_3), 0.92 (3H, s, 19-H_3), 0.95 (3H, t, *J*=7.3 Hz, 29-H_3), 1.05 (3H, d, *J*=6.4 Hz, 21-H_3), 2.61 (1H, dd, *J*=12.2 and 3.9 Hz, 5-H), 2.24 (1H, dd, *J*=12.7 and 4.4 Hz, 7-H); ^{13}C NMR (150 MHz, C_6D_6): δ -2.64 (3 x Si<u>C</u>H_3), 11.82 (C-18), 12.21 (C-29), 15.83 (C-19), 19.01 (C-21), 19.24 and 20.03 (C-26 and C-27), 22.76 (C-2), 22.94 (C-11), 23.53 (C-28), 25.40 (C-4), 25.76 (C-3), 25.95 (C-15), 26.54 (C-23), 28.08 (C-16), 29.59 (C-25), 30.43 (C-7a), 34.31 (C-22), 36.58 (C-20), 38.63 (C-10), 40.29 (C-12), 41.61 (C-8), 41.72 (C-1), 42.27 (C-13), 46.29 (C-24), 50.67 (C-7), 54.77 (C-5), 56.34 (C-14), 56.63 (C-17), 59.64 (C-9), 212.79 (C-6).
10. The sterically disadvantageous axial attack of carbanions to cyclohexanones like this is well documented in organoaluminum Lewis acid-promoted alkylation, see Maruoka, K.; Itoh, T.; Sakurai, M.; Nonoshita, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1988**, *110*, 3588-3597.
11. For the stereoelectronic requirements for the Tiffeneau-Demjanov type rearrangements, see ref. 6 and Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*, Pergamon Press, Oxford, 1983; pp. 163-208; and references cited therein.
12. Takatsuto, S.; Yazawa, N.; Ishiguro, M.; Morisaki, M.; Ikekawa, N. *J. Chem. Soc., Perkin Trans. 1* **1984**, 139-146.
13. Watanabe, T.; Kuriyama, H.; Furuse, T.; Kobayashi, K.; Takatsuto, S. *Agric. Biol. Chem.* **1988**, *52*, 2117-2118.
14. All new compounds were fully characterized by ^{1}H and ^{13}C NMR and MS spectra. Selected ^{1}H NMR data of compounds **2**, **14** and **15** are shown here. **2** (600 MHz, $CDCl_3$-CD_3OD, 10:1): δ 0.65 (3H, s, 18-H_3), 0.77 (3H, s, 19-H_3), 0.84 (3H, d, *J*=6.8 Hz, 28-H_3), 0.88 (3H, d, *J*=6.8 Hz, 21-H_3), 0.94 and 0.96 (each 3H, each d, *J*=6.8 Hz, 26- and 27-H_3), 2.29 (1H, ddd, *J*=17.6, 12.7 and 4.9 Hz, 6aβ-H), 2.46 (1H, ddd, *J*=17.6, 4.4 and 2.9 Hz, 6aα-H), 3.26 (1H, dd, *J*=12.2 and 3.4 Hz, 5-H), 3.52 (1H, br d, *J*=8.3 Hz, 22-H), 3.59 (1H, m, 2-H), 3.68 (1H, dd, *J*=8.3 and 1.0 Hz, 23-H), 3.94 (1H, m, 3-H); **14** (600 MHz, $CDCl_3$-CD_3OD, 10:1): δ 0.69 (3H, s, 18-H_3), 0.81 (3H, s, 19-H_3), 0.84 (3H, d, *J*=6.8 Hz, 28-H_3), 0.88 (3H, d, *J*=6.8 Hz, 21-H_3), 0.94 and 0.96 (each 3H, each d, *J*=6.8 Hz, 26- and 27-H_3), 2.20 and 2.31 (each 1H, each m, 6-H_2), 2.27 (1H, m, 5-H), 2.36 (1H, d, *J*=12.2 Hz, 7β-H), 2.53 (1H, dd, *J*=12.2 and 12.2 Hz, 7α-H), 3.52 (1H, br d, *J*=8.3 Hz, 22-H), 3.68 (1H, dd, *J*=8.3 and 1.5 Hz, 23-H), 3.59 (1H, ddd, *J*=12.2, 4.4 and 3.4 Hz, 2-H), 3.88 (1H, m, 3-H); **15** (300 MHz; $CDCl_3$) δ 0.72 (3H, s, 18-H_3), 0.85 (3H, d, *J*=6.9 Hz, 21- or 28-H_3), 0.88 (3H, d, *J*=6.4 Hz, 21- or 28-H_3), 0.95 (3H, d, *J*=6.4 Hz, 26- or 27-H_3), 0.97 (3H, d, *J*=6.3 Hz, 26- or 27-H_3), 1.12 (3H, s, 19-H_3), 3.38 (1H, br d, *J*=4.0 Hz, 5-H), 3.53 (1H, m, 2-H), 3.53 (1H, br d, *J*=8.5 Hz, 22-H), 3.71 (1H, dd, *J*=8.5 and 1.5 Hz, 23-H), 3.82 (1H, m , 3-H), 5.70 (1H, d, *J*=8.4 Hz, 3-OH, disappeared by addition of D_2O).