Organic & Biomolecular Chemistry

COMMUNICATION

View Article Online
View Journal

Cite this: DOI: 10.1039/c7ob01400g

Received 9th June 2017,
Accepted 7th August 2017

DOI: 10.1039/c7ob01400g

rsc.li/obc

7-Step total synthesis of (+)-EBC-329: Photoisomerisation reveals new *seco*-casbane family member†

Timothy J. Vanden Berg, David M. Pinkerton and Craig M. Williams *

The first *seco*-casbane, EBC-329, isolated from the Australian rainforest, was synthesised from (+)-2-carene in seven steps. This endeavour not only established the absolute stereochemical assignment as (8*R*,9*S*)-EBC-329, but also identified, *via* photoisomerisation, a new *seco*-casbane family member.

Casbanes are considered a rare family of *gem*-dimethylcyclopropyl diterpene macrocycles.[1,2] The parent skeletal hydrocarbon, casbene (**1**) (Fig. 1), was the first family member to be reported,[3] and since then casbanes have been found in both terrestrial[4] and marine[5] organisms. Our investigation into the Australian rainforest plant *Croton insularis*[6] has revealed various casbanes [*e.g.* EBC-181 (**2**),[7] EBC-304 (**3**)[8] and EBC-324 (**4**)[9]], and the first *seco*-casbanes 328 (**5**),[10] 329 (**6**)[9] and 363 (**7**)[10] (Fig. 1).

Surprisingly, casbanes have received little attention in terms of total synthesis, especially given that they display a range of biological activities.[1,4,5,7,8] Crombie and Pattenden were the first to synthesise casbene (**1**),[11] soon followed by Takahashi,[12] and later by McMurry.[13] However, no further total syntheses of casbanes have surfaced since these reports in the 1980s. In comparison, the *seco*-casbane EBC-329 (**6**) succumbed to total synthesis in 2015,[14] one year after we disclosed the structure.[9] The Thombal and Jadhav synthesis of racemic **6** was achieved in 13 steps with a reported overall yield of 10%; however, the supplied spectroscopic evidence was inconsistent with that of the natural product (see ESI†).

Inspired to unambiguously determine the absolute stereochemistry of EBC-329, and to further pursue its anti-cancer properties (*e.g.* IC_{50} of 0.6 μM against the K562 chronic myelogenous leukemia cell line[15]), we embarked on a synthetic campaign.

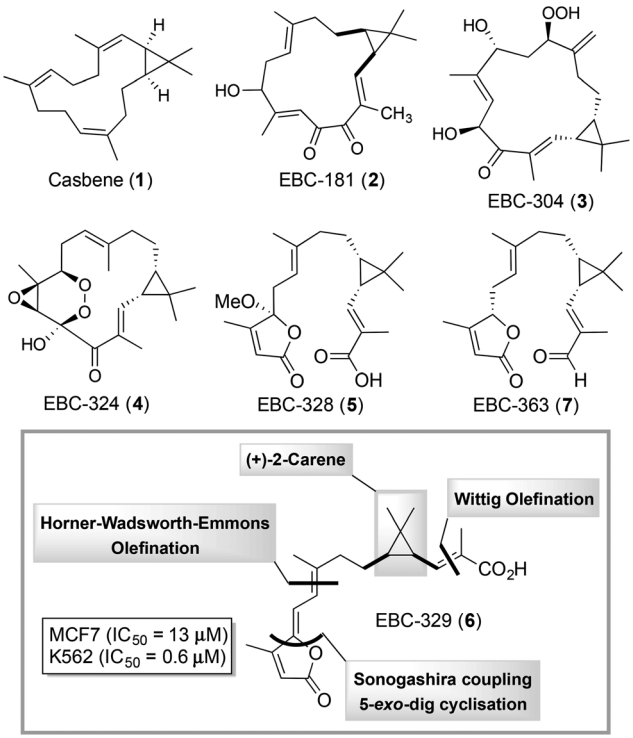


Fig. 1 Casbanes **1**–**7**, and retrosynthetic analysis of EBC-329 (**6**).

Herein is reported a 7-step total synthesis of EBC-329 starting from the chiral pool.

As EBC-329 (**6**) contains a *gem*-dimethylcyclopropane with two embedded stereocentres, it was difficult to surpass the chiral pool in the retrosynthetic analysis (Fig. 1), especially considering the chiral pool was successfully exploited for the synthesis of casbene (**1**).[11–13] In this regard, commercially available (+)-2-carene (**8**) was selected, even though it was likely to lead to the minor (or non-natural) enantiomer based on the cyclopropane stereochemical assignments seen in **1**, **3**, **4**, **5** and **7**. That being said, there is evidence that the absolute configuration of casbane cyclopropane units can vary within the same organism.[5b]

School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, 4072, Australia. E-mail: c.williams3@uq.edu.au

†Electronic supplementary information (ESI) available: Experimental procedures, compound characterisation, X-ray crystallography and copies of ^{1}H and ^{13}C NMR, UV-Vis and UV-Vis circular dichroism spectra. CCDC 1521107. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c7ob01400g

It was envisaged that oxidative ring opening of (+)-2-carene (**8**) would provide differentiated carbonyl groups. Such an approach would allow judicious choice in chemoselective methods for installing the required unsaturation. Butenolide functionality could be installed sequentially or deployed in a concerted fashion using a Sonogashira[16]/cyclisation[17] strategy (Fig. 1).

Attempts to procure ketoaldehyde **9** *via* ozonolysis[13] of **8** were unsatisfactory, thus prompting the use of a two-step procedure involving dihydroxylation followed by oxidative cleavage (Scheme 1).[18] Ketoaldehyde **9** was then subjected to Wittig olefination with known phosphorane **10**, generating ketoester **11** with exclusive *E* selectivity (determined through an NOE difference experiment, see ESI†) (Scheme 1). The overall yield of **11** from **8** over three steps was enhanced to 52% if ketoaldehyde **9** was not purified prior to Wittig olefination. Ketoaldehyde **9** was prone to oxidation in the open atmosphere, as has been previously reported.[19]

Initially, a Petersen olefination[20] strategy was pursued to access enyne **12**. Although this was productive, both standard and modified conditions provided unfavourable *E*/*Z*-isomer ratios of **12** (*i.e.* the unwanted 12*Z*-**12** as the major isomer). Fortunately, resorting to the Horner–Wadworth–Emmons olefination protocol, using the known diisopropyl phosphonate **13**,[21] installed the enyne motif in a favourable 2.4 : 1.0 *E* : *Z* ratio (see below). TBAF-mediated protodesilylation was subsequently performed *in situ* to furnish enyne **14**, followed by hydrolysis of the methyl ester to yield the corresponding acid **15** in 63% yield over three steps (Scheme 1).

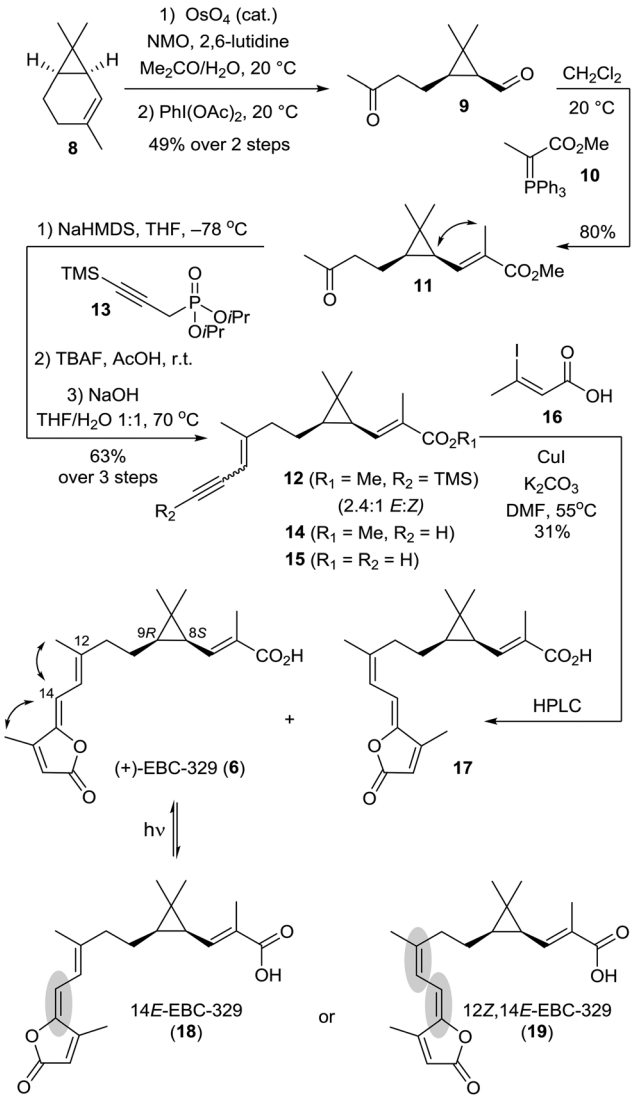
The ester protection was removed prior to the installation of the butenolide because prior work in our group revealed the methyl ester of EBC-329 to be unstable. The status of the carboxyl group (*i.e.* ester or free acid) did not appear to have an effect on the outcome of the butenolide installation.

The final step of the synthesis required the installation of the γ-alkylidenebutenolide. This was accomplished in 31% yield with exclusive *Z*-selectivity (*i.e.* both isomers are 14*Z*) using iodoacrylic acid **16**,[22] following previously reported one pot palladium-free conditions.[23] An NOE difference experiment supported the butenolide configuration assigned to **6** (see ESI†). Integration of the ^{1}H NMR spectrum recorded on the butenolide mixture (*i.e.*, **6** and **17**) confirmed that the 12*E*/12*Z* ratio was unvaried in the reactions subsequent to the Horner–Wadsworth–Emmons olefination. Unfortunately, NOE experiments performed on **12** through to **15** were unproductive. The two butenolide diastereomers were then separated by normal-phase HPLC to yield pure EBC-329 (**6**) and impure 12*Z*-EBC-329 (**17**) (Scheme 1), which could not be further purified.

The specific rotation obtained for synthetic 8*S*,9*R*-EBC-329 (**6**) was opposite to that reported for the natural sample, confirming the absolute stereochemistry of natural (−)-EBC-329 (**6**) as 8*R*,9*S*. This assignment was supported by comparison between the UV-Vis circular dichroism spectra of synthetic and natural EBC-329 (see ESI†).

As photoisomerisations of alkylidenebutenolides are precedented,[24–26] we exposed a deuterochloroform solution of synthetic EBC-329 to laboratory fluorescent lighting for 60 h. A new compound was generated which seemed to be in steady-state equilibrium with EBC-329. No conversion was observed in the absence of light under identical conditions. Based on ^{1}H NMR spectral comparisons to both EBC-329 and 12*Z*-EBC-329 (**17**), the photochemical product was assigned as one of either 14*E*-EBC-329 (**18**) or 12*Z*,14*E*-EBC-329 (**19**) (Scheme 1). Unambiguous assignment of the photoisomer's stereochemistry could not be made with the available NMR data. The ^{1}H NMR spectrum of natural EBC-329 [9] was then re-evaluated to reveal this same photoisomer as a minor co-isolate (see ESI†). Interestingly, the ratio of isomers observed in the natural and irradiated synthetic samples were comparable.

On this basis, the photoisomer was identified as a new member of the *seco*-casbane class of natural products, and it



Scheme 1 Total synthesis of (+)-EBC-329. Double-headed arrows depict key NOE observations.

was tentatively assigned the same absolute configuration as co-isolated EBC-329 (*i.e.* 8*R*,9*S*). Although artefacts of isolation may arise through photoisomerisation, the typical exposure of Northern Australian terrestrial plants to prolonged, intense sunlight supports the natural product status of the photoisomer.

In conclusion, the synthesis of (+)-(8*S*,9*R*)-EBC-329 (**6**) was achieved in a concise and efficient manner, culminating in a seven step sequence. The synthetic approach described herein is a substantial improvement over that previously reported, and confirms for the first time the absolute stereochemistry of naturally-occurring (−)-EBC-329 (**6**) as 8*R*,9*S*. In addition, photoisomerisation of the final product led to the identification of either 8*R*,9*S*-14*E*-EBC-329 (**18**) or 8*R*,9*S*-12*Z*-14*E*-EBC-329 (**19**) as a new *seco*-casbane natural product.

The authors thank EcoBiotics Ltd and the University of Queensland (UQ) for financial support. We gratefully acknowledge Dr S. Chow and Ms Y. P. Tan for assistance with HPLC, Prof. P. V. Bernhardt for assistance with collecting X-ray crystallographic data, and Dr A. Savchenko for helpful discussions on structure elucidation by NMR. T. J. V. thanks the Australian Government for a Research Training Program Scholarship. The Australian Research Council for a Future Fellowship award (grant number FT110100851) to C. M. W. is also gratefully acknowledged.

Conflicts of interest

T. J. V. and D. M. P. declare no conflicts of interest. C. M. W. is a consultant to EcoBiotics Ltd.

Notes and references

1 M. J. Durán-Peña, J. M. Botubol Ares, I. G. Collado and R. Hernández-Galán, *Nat. Prod. Rep.*, 2014, **31**, 940–952.
2 Y.-H. Choi, J. Kim, J. M. Pezzuto, A. D. Kinghorn, N. R. Farnsworth, H. Latter and H. Wagner, *Tetrahedron Lett.*, 1986, **27**, 5795–5798.
3 D. R. Robinson and C. A. West, *Biochemistry*, 1970, **9**, 70–79.
4 See for example: (*a*) Q.-L. Liang, C.-C. Dai, J.-H. Jiang, Y.-P. Tang and J.-A. Duan, *Fitoterapia*, 2009, **80**, 514–516; (*b*) F.-G. Shao, R. Bu, C. Zhang, C.-J. Chen, J. Huang and J.-H. Wang, *J. Asian Nat. Prod. Res.*, 2011, **13**, 805–810; (*c*) W.-W. Tao, J.-A. Duan, Y.-P. Tang, N.-Y. Yang, J.-P. Li and Y.-F. Qian, *Phytochemistry*, 2013, **94**, 249–253; (*d*) F. A. e Silva Filho, J. N. da Silva Jr., R. Braz-Filho, C. A. de Simone, E. R. Silveira and M. A. Sousa Lima, *Helv. Chim. Acta*, 2013, **96**, 1146–1154.
5 See for example: (*a*) C.-X. Zhang, S.-J. Yan, G.-W. Zhang, W.-G. Lu, J.-Y. Su, L.-M. Zeng, L.-Q. Gu, X.-P. Yang and Y.-J. Lian, *J. Nat. Prod.*, 2005, **68**, 1087–1089; (*b*) Y. Li, M. Carbone, R. M. Vitale, P. Amodeo, F. Castelluccio, G. Sicilia, E. Mollo, M. Nappo, G. Cimino, Y.-W. Guo and M. Gavagnin, *J. Nat. Prod.*, 2010, **73**, 133–138; (*c*) J. Yin, M. Zhao, M. Ma, Y. Xu, Z. Xiang, Y. Cai, J. Dong, X. Lei, K. Huang and P. Yan, *Mar. Drugs*, 2013, **11**, 455–465; (*d*) M.-E. F. Hegazy, T. A. Mohamed, A. I. Elshamy, M. A. Al-Hammady, S. Ohta and P. W. Paré, *Molecules*, 2016, **21**, 308.
6 For previous work on this plant system by our group see: (*a*) L. A. Maslovskaya, A. I. Savchenko, V. A. Gordon, P. W. Reddell, C. J. Pierce, P. G. Parsons and C. M. Williams, *Org. Lett.*, 2011, **13**, 1032–1035; (*b*) L. A. Maslovskaya, A. I. Savchenko, V. A. Gordon, P. W. Reddell, C. J. Pierce, P. G. Parsons and C. M. Williams, *Aust. J. Chem.*, 2015, **68**, 652–659; (*c*) L. A. Maslovskaya, A. I. Savchenko, C. J. Pierce, V. A. Gordon, P. W. Reddell, P. G. Parsons and C. M. Williams, *Chem. – Eur. J.*, 2014, **20**, 14226–14230; (*d*) L. A. Maslovskaya, A. I. Savchenko, V. A. Gordon, P. W. Reddell, C. J. Pierce, P. G. Parsons and C. M. Williams, *RSC Adv.*, 2016, **6**, 25110–25113.
7 L. A. Maslovskaya, A. I. Savchenko, E. H. Krenske, C. J. Pierce, V. A. Gordon, P. W. Reddell, P. G. Parsons and C. M. Williams, *Angew. Chem., Int. Ed.*, 2014, **53**, 7006–7009.
8 L. A. Maslovskaya, A. I. Savchenko, V. A. Gordon, P. W. Reddell, C. J. Pierce, P. G. Parsons and C. M. Williams, *Chem. – Eur. J.*, DOI: 10.1002/chem.201604674.
9 L. A. Maslovskaya, A. I. Savchenko, E. H. Krenske, V. A. Gordon, P. W. Reddell, C. J. Pierce, P. G. Parsons and C. M. Williams, *Chem. Commun.*, 2014, **50**, 12315–12317.
10 L. A. Maslovskaya, A. I. Savchenko, V. A. Gordon, P. W. Reddell, C. J. Pierce, P. G. Parsons and C. M. Williams, *Eur. J. Org. Chem.*, 2016, 1673–1677.
11 (*a*) L. Crombie, G. Kneen and G. Pattenden, *J. Chem. Soc., Chem. Commun.*, 1976, 66–68; (*b*) L. Crombie, G. Kneen, G. Pattenden and D. Whybrow, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1711–1717.
12 K. Toma, E. Miyazaki, T. Murae and T. Takahashi, *Chem. Lett.*, 1982, 863–864.
13 J. E. McMurry and G. K. Bosch, *J. Org. Chem.*, 1987, **52**, 4885–4893.
14 R. S. Thombal and V. H. Jadhav, *Org. Biomol. Chem.*, 2015, **13**, 9485–9491.
15 C. B. Lozzio and B. B. Lozzio, *Blood*, 1975, **45**, 321–334.
16 R. Chinchilla and C. Nájera, *Chem. Rev.*, 2007, **107**, 874–922.
17 (*a*) T. M. Ugurchieva and V. V. Veselovsky, *Russ. Chem. Rev.*, 2009, **78**, 337–373; (*b*) D. W. Knight, *Contemp. Org. Synth.*, 1994, **1**, 287–315.
18 K. C. Nicolaou, V. A. Adsool and C. R. H. Hale, *Org. Lett.*, 2010, **12**, 1552–1555.
19 G. B. Hammond, M. B. Cox and D. F. Wiemer, *J. Org. Chem.*, 1990, **55**, 128–132.
20 (*a*) D. J. Peterson, *J. Org. Chem.*, 1968, **33**, 780–784; (*b*) L. F. van Staden, D. Gravestock and D. J. Ager, *Chem. Soc. Rev.*, 2002, **31**, 195–200, and references therein; (*c*) Y. Yamakado, M. Ishiguro, N. Ikeda and H. Yamamoto, *J. Am. Chem. Soc.*, 1981, **103**, 5568–5570.

21 (*a*) For the synthesis of **13** see: G. Yao and K. Steliou, *Org. Lett.*, 2002, **4**, 485–488. Conditions used for the HWE olefination see: K. M. Brummond, J. Lu and J. Petersen, *J. Am. Chem. Soc.*, 2000, **122**, 4915–4920.

22 (*a*) B. Wang, C. Lu, S.-Y. Zhang, G. He, W. A. Nack and G. Chen, *Org. Lett.*, 2014, **16**, 6260–6263; (*b*) The first X-ray crystal structure of iodoacrylic acid **16** is reported herein, see ESI. CCDC 1521107.†

23 S. Inack-Ngi, R. Rahmani, L. Commeiras, G. Chouraqui, J. Thibonnet, A. Duchêne, M. Abarbri and J.-L. Parrain, *Adv. Synth. Catal.*, 2009, **351**, 779–788.

24 For γ,δ-photoisomerisation of a γ-alkenylbutenolide, see: (*a*) S. D. Townsend and G. A. Sulikowski, *Org. Lett.*, 2013, **15**, 5096–5098; (*b*) K. Sastraruji, S. G. Pyne, A. T. Ung, P. Mungkornasawakul, W. Lie and A. Jatisatienr, *J. Nat. Prod.*, 2009, **72**, 316–318.

25 For combined γ,δ- and ε,ζ-photoisomerisation of a γ-alkylidenebutenolide, see: T. Kajikawa, S. Hasegawa, T. Iwashita, T. Kusumoto, H. Hashimoto, D. M. Niedzwiedzki, H. A. Frank and S. Katsumura, *Org. Lett.*, 2009, **11**, 5006–5009.

26 For photoisomerisation at even more remote alkenes in a conjugated γ-alkylidenebutenolide, see: T. Kajikawa, K. Aoki, R. S. Singh, T. Iwashita, T. Kusumoto, H. A. Frank, H. Hashimoto and S. Katsumura, *Org. Biomol. Chem.*, 2009, **7**, 3723–3733.

