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(+)-Brevione A. The first member of a novel family of bioactive spiroditerpenoids isolated from *Penicillium brevicompactum* Dierckx[1]

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Abstract

(+)-Brevione A, the first member of a novel family of bioactive spiroditerpenoids, a potential allelopathic agent, has been isolated from the ethyl acetate active fractions of the aqueous acetone extracts of semi-solid fermented *Penicillium brevicompactum* Dierckx. The structure displays the novel spiroditerpenoid skeleton of breviane. The structure elucidation of brevione A was performed by homo- and hetero-nuclear 2D NMR spectral data. On the basis of combined studies of the theoretical conformations and NOEDIFF data, its relative stereochemistry is proposed.

Keywords: biologically active compound; allelochemical; terpenoid; *Penicillium brevicompactum*.

The discovery of new allelochemicals from plants or microbes has attracted much attention in the last 20 years. Allelochemicals have been implicated as biocommunicators,[2] and are potential sources of new structural types of pesticides with new modes of action which may be less harmful than those presently used in agriculture,[3] as well as new pharmaceuticals. Allelopathy,[4] an emerging branch of applied sciences which studies biochemical plant–plant and plant–microorganism interactions, may help in providing new generations of natural phytotoxins and mycotoxins as models for natural agrochemicals and pharmaceuticals.

The genus *Penicillium* is well-known for producing a variety of bioactive metabolites, possessing a wide variety of biological properties, including plant growth regulators,[5] and especially *P. brevicom-*

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pactum which produce polyketides, peptides and a collection of diverse heterocyclic compounds with pyrrole, pyrroline and oxazine structures[6] that show several biological activities such as anti-juvenile-hormones, insecticidal and fungicidal activity, and as potent agonists on the D-*myo*-inositol-1,4,5-triphosphate receptors.[7] During the course of examining fungi for biologically active natural products, we accessed a strain of *P. brevicompactum* from leaf litter collected in the Waipona Forest, New Zealand. However, in this strain the penicilli were atypically irregular, thus the assignments of the species name is provisional. Upon subsequent semi-solid fermentation, compound **1** was isolated from the ethyl acetate active fraction using an etiolated wheat coleoptile bioassay to detect activity in the crude extract and to direct the fractionation.[8] This extract afforded the novel brevione A (**1**) which contains a novel spiroditerpenoid skeleton (Fig. 1).

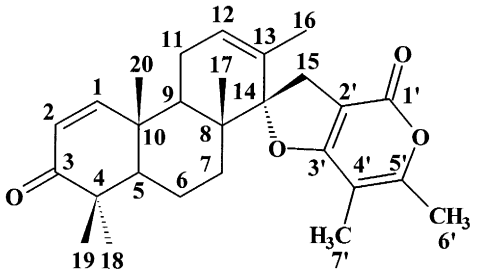

Fig. 1. Brevione A

Brevione A (**1**) gave a HREIMS spectrum with a molecular ion at *m/z* 423.2504 $[M+1]^+$ corresponding to a molecular formula $C_{27}H_{34}O_4$ (calculated 423.2423 for $[M+1]^+$ corresponding to $C_{27}H_{35}O_4$). The IR spectrum showed absorptions at 1714 and 1673 cm^{-1} in accord with the presence of two α,β-carbonyl systems in the molecule. The ^1H NMR spectrum[9] had two deshielded doublets (H-1, δ 7.07 d, $J_{1,2}$=10.2 Hz and H-2; δ 5.85 d, $J_{2,1}$=10.2 Hz) corresponding to two olefinic protons placed on a *cis*-double bond conjugated with a carbonyl group.The ^1H–^1H–DQF–COSY showed correlation between a broad doublet signal (H-12; δ 5.70 brd, $J_{12,11}$=5.3 Hz) and a singlet (H-16; δ 1.65 brs) and methylene group signals (H-11; δ 2.27 and 2.14), and between one of the protons on C-11 and a doublet of doublets (H-9; δ 1.95). Signals of an isolated methylene group appeared at δ 3.05 (H-15a, d, $J_{15a,15b}$=15.8 Hz) and δ 2.90 (H-15b, d), and signals for a CH-CH_2-CH_2 moiety appeared at δ 1.38–1.59. Interpretation of these data led to the following three partial structures: C-1 to C-2, C-5 to C-7, and C-9 to C-16 (Fig. 2). In addition, the ^1H NMR spectrum showed six methyl signals (δ 0.99, 1.10, 1.13, 1.18, 1.91, and 2.21). The ^{13}C NMR spectrum[9] confirmed the presence of a carbonyl group C-3 (δ 204.7), an ester group C-1′ (δ 170.9), two quaternary double bonds connected to oxygen from C-2′ to C-5′ (δ 161.8; 160.4; 102.7 and 99.3), two double bonds, one conjugated to a carbonyl group (C-1, δ 157.7; C-2, δ 125.5) and one not thus conjugated (C-12; δ 127.2 and C-13, δ 132.1). The HMQC and HMBC spectra led to the complete assignment of the ^{13}C NMR spectrum, the HMBC correlations connected the diterpene-like part of compound **1** (Fig. 2). The right part of compound **1** was established as an α,β,γ,δ-unsaturated δ-lactone following the observed correlations in HMBC of H-15, H-6′ and H-7′ signals with the five quaternary carbons C-1′ to C-5′. The HMBC and NOESY correlations of H-15a and H-15b are the keys to connecting the two pieces of compound **1** and to obtaining its structure (Fig. 2).

The relative stereochemistry was assigned on the basis of a NOESY experiment (Fig. 3), where the observed effects between H-15a and H-7, and H-17; H-15b and H-16 but small with H-17, clearly establish a relative β orientation for the spiranic methylene; and those observed between H-20 and H-11α and H-1; H-11β and H-17 and H-20; H-9 and H-5; as well as between H-6′ and H-7′ corroborate the typical relative stereochemistry of the diterpene moiety of **1**.

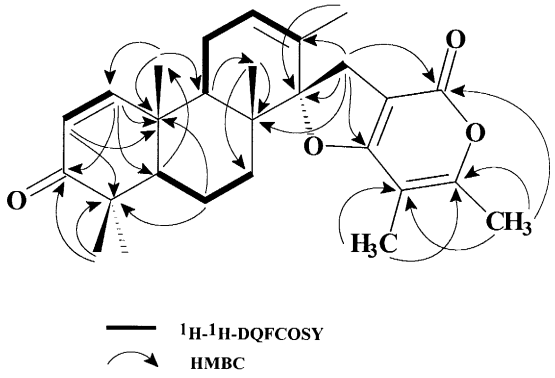

Fig. 2. Structure and selected 2D NMR correlations of brevione A (**1**)

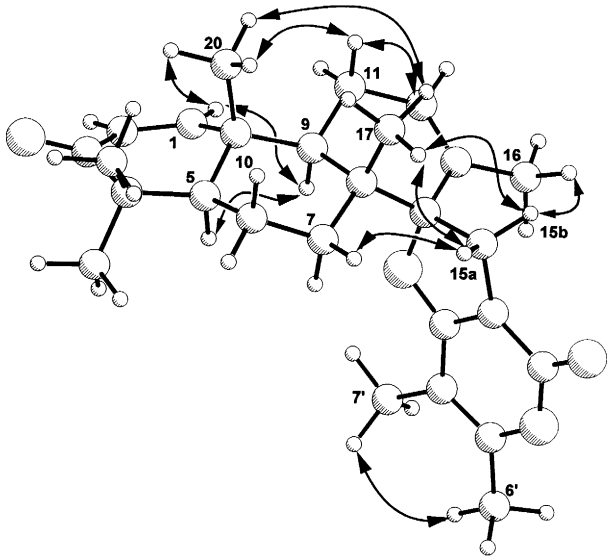

Fig. 3. Observed NOEs for the most stable conformer of **1** using PM3 calculations

Both, the stereochemistry and the presence of a spirane ring are corroborated with good agreement observed between the angles found for the most stable conformer obtained by using PM3 calculations[10] and the experimental coupling constants.[9]

This compound is of particular interest since it is the first member of a novel bioactive spiroditerpenoid skeleton for which we suggest the name breviane. Brevione A inhibits wheat coleoptile growth 38% at 1 mM ($P<0.01$). Its biogenesis may proceed through combined biogenesis from polyketide and terpenoid pathways. Some other examples of metabolites from this combined biogenesis have been previously reported from *P. brevicompactum*.[11]

Acknowledgements

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References

1. Continuation in the series ‘Allelochemicals from New Zealand fungi’.
2. (a) Fischer, N. H. In *The Science of Allelopathy*; Putnam, A. R., Tang, C. S., Eds.; John Wiley & Sons: New York, USA, 1986; Chapter 12, pp. 203–218.(b) Macías, F. A.; Oliva, R. M.; Simonet, A. M; Galindo, J. C. G. In *Allelopathy in Rice*; Oloffdoter, M. Ed.; I.R.R.I. Press: Los Baños, Philippines, 1998; Chapter 7, pp. 69–79.(c) Harborne, J. B. *Nat. Prod. Report* **1993**, *10*, 327. (d) Harborne, J. B. *Nat. Prod. Report* **1997**, *14*, 83.
3. (a) Duke, S. O. *Reviews of Weed Science* **1986**, 2, 15. (b) Macías, F.A. In *Allelopathy: Organisms, Processes, and Applications*; Inderjit, Dakshini, K. M. M., Einhellig, F. A., Eds.; ACS Symposium Series, 582: Washington, D.C., USA, 1995; Chapter 23, pp. 310. (c) Rice, E. L. *Biological Control of Weeds and Plant Diseases*; University of Oklahoma Press: Norman, USA, 1995.
4. (a) Rice, E. L. *Allelopathy*; 2nd ed.; Academic Press: New York, USA, 1984. (b) Allelopathy is officially defined as: The science that studies any process involving, mainly, secondary metabolites produced by plants, algae, bacteria, and fungi that influence the growth and development of agricultural and biological systems, including positive and negative effects, IAS International Allelopathy Society Constitution (1996), First World Congress on Allelopathy. A Science for the Future, September, Cádiz, Spain.
5. (a) Matsuzaki, K.; Takara, H.; Inokoshi, J.; Tanaka, H.; Masuma, R.; Omura, S. *J. Antibiot.* **1998**, *51*, 1004–1011. (b) Tomoda, H.; Tabata, N. Masuma, R.; Si, S. Y.; Omura, S. *J. Antibiot.* **1998**, *51*, 618–623. (c) Medentsev, A. G.; Akimenko, V. K. *Phytochemistry* **1998**, *47*, 935–959. (d) Kimura, Y.; Tani, K.; Kojima, A.; Sotoma, G.; Okada, K.; Shimada, A. *Phytochemistry* **1996**, *41*, 665–669.
6. (a) Canonica, L.; Kroszcynski, W.; Ranzi, B. M.; Rindone, B.; Scolastico, C. *J. Chem. Soc., Chem. Commun*. **1971**, *6*, 257– 260 (b) Moya, P.; Cantin, A.; Castillo, M. A.; Primo, J.; Miranda, M. A.; Priomoyufera, E. *J. Org. Chem*. **1998**, *63*, 8530–8535. (c) Cantin, A.; Moya, P.; Castillo, M. A.; Primo, J.; Miranda, M. A.; Primoyufera, E. *Europ. J. Org. Chem*. **1999**, *1*, 221–226.
7. (a) Castillo, M. A.; Moya, P.; Cantin, A.; Miranda, M. A.; Primo, J.; Hernández, E.; Primoyufera, E. J. *Agric. Food Chem*. **1999**, *47*, 2120–2124. (b) Cantin, A.; Moya, P.; Miranda, M. A.; Primo, J.; Primoyufera, E. *J. Agric. Food Chem*. **1998**, *46*, 4748–4753. (c) Murphy, C. T.; Riley, A. M.; Lindley, C. J.; Jenkins, D. J.; Westwick, J.; Potter, B. V. L. *Molecular Pharm*. **1997**, *52*, 741–748. (d) Jenkins, D. J.; Potter, B. V. L. *Carbohydrate Research* **1996**, *287*, 169–182.
8. (a) Cutler, H. G.; Himmelsbach, D. S.; Yagen, B.; Arrendale, R. F.; Jacyno, J. M.; Cole, P. D.; Cox, R. H. *J. Agric. Food. Chem*. **1991**, *39*, 977–980. (b) Parker, S. R.; Cutler, H. G.; Schreiner, P. R. *Biosci. Biotech. Biochem*. **1995**, *59*, 1126–1127. (c) Parker, S. R.; Cutler, H. G.; Schreiner, P. R. *Biosci. Biotech. Biochem*. **1995**, *59*, 1747–1749. (d) Cutler, H. G.; Cutler, S. J.; Ross, S. A.; El Sayed, K.; Dugan, F. M.; Barlett, M. G.; Hill, A. A.; Hill, R. A.; Parker, S. R. *J. Nat. Prod*. **1999**, *62*, 137–139.
9. Colorless oil; $[\alpha]_D^{25}$=+111 ($CHCl_3$, c=0.1); IR $_{max}$ (neat, KBr) cm^{-1}: 2927, 2869, 1714, 1674 (C=C–C=O), 1574 (C=C); 1274 (ether); δ_H (400 MHz, $CDCl_3$): 7.07 (1H, d, J 10.2 Hz, H-1), 5.85 (1H, d, J 10.2 Hz, H-2), 5.70 (1H, brd, J 5.1 Hz, H-12), 3.05 (1H, d, J 15.8 Hz, H-15a), 2.90 (1H, d, J 15.8 Hz, H-15b), 2.27 (1H, ddd, J 17.7; 5.1; 5.1 Hz, H-11α), 2.21 (3H, s, H-7′), 2.14 (1H, brdd, J 17.7; 12.0 Hz, H-11β), 1.95 (1H, dd, J 12.0; 5.1 Hz, H-9α), 1.91 (3H, s, H-6′), 1.65 (3H, brs, H-16), 1.61 (1H, m, H-7β), 1.58 (3H, m, H-5α, H-6α, H-6β), 1.38 (1H, m, H-7α), 1.18 (3H, s, H-20), 1.13 (3H, s, H-18*), 1.10 (3H, s, H-19*), 0.99 (3H, s, H-17), *may be interchanged ; δ_C (125.772 MHz, $CDCl_3$ centred at 77.0 ppm): 204.7 (C-3); 170.9 (C-1′); 161.8 (C-3′); 160.4 (C-5′); 157.7 (C-1); 132.1 (C-13); 127.2 (C-12); 125.5 (C-2); 102.7 (C-4′); 99.3 (C-14); 99.3 (C-2′); 53.0 (C-5); 44.4 (C-4); 42.1 (C-9); 41.3 (C-8); 38.9 (C-10); 31.9 (C-7); 28.5 (C-15); 27.7 (C-18*); 22.8 (C-11); 21.5 (C-19*); 18.9 (C-20); 18.2 (C-6); 18.2 (C-16); 17.1 (C-7′); 16.3 (C-17); 9.5 (C-6′); EIMS, *m/z* (rel. int.): 422 $[M]^+$ (100), 407 $[M-CH_3]^+$ (14.3), 380 $[M-42]^+$(13.9), 379 $[M-43]^+$(3.3), 351 $[M-71]^+$ (6.2), 350 $[M-72]^+$(3.3), 335 (3.5), 324 (3.5), 295 (2.5), 285 (3.8), 269 (14.9), 218 (11.2). HREIMS calcd for $C_{27}H_{35}O_4$: 423.2423, found: 423.2504 $[M+1]^+$.
10. Stewart, J. J. P. *J. Comput. Chem*. **1989**, *10*, 209. Molecular orbital calculations were carried out using PM3 Hamiltonian as implemented in MOPAC 6.0. Geometries were fully optimized using the PRECISE keyword.
11. Arogozzini, F.; Colombo, L.; Gennari, C.; Gualandris, R.; Potenza, D.; Scolastico, C. *J. Chem. Soc., Perkin Trans.1* **1982**, 365–373.