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A CAROTANE SESQUITERPENE AS A POTENT MODULATOR OF THE MAXI-K CHANNEL FROM *ARTHRINIUM PHAESOSPERMUM*

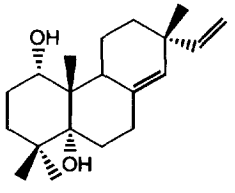
John G. Ondeyka*, Richard G. Ball, Maria L. Garcia, Anne W. Dombrowski, Gloria Sabnis, Gregory J. Kaczorowski, Deborah L. Zink, Gerald F. Bills, Michael A. Goetz, William A. Schmalhofer, Sheo B.Singh

Merck Research Laboratories, P.O. Box 2000
Rahway, N.J. 07065 (USA)

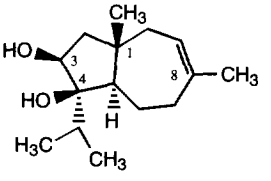
Abstract. A potent modulator of the Maxi-K channel, isolated from *Arthrinium phaeospermum*, has been determined to be a carotane sesquiterpene by spectroscopic means. X-ray crystallographic studies demonstrated this compound as being identical to CAF-603, previously discovered as an antifungal agent.

The large conductance, calcium-activated potassium (Maxi-K) channel is a member of a large family of proteins present in neuronal and smooth muscle tissue.[1] Elevation of intracellular calcium is required for neurotransmitter release and smooth muscle contraction. Agonists of Maxi-K may therefore be useful in treatment of neurological disorders such as neural ischemia and airway smooth muscle disorders such as asthma.[2] In contrast, inhibitors of this channel might be potentiators of neurotransmitter release. The channel can be potently blocked by scorpion venom peptides, such as charybdotoxin (ChTX), and its physiological role can be evaluated with modulators of ChTX binding.[3,4]

Natural products screening of fermentation extracts using [^{125}I] ChTX binding to Maxi-K channels in bovine aortic sarcolemmal membrane vesicles has previously led to the discovery of a Maxi-K agonist, maxikdiol, isolated from an unidentified coelomycete.[5] The methylethyl ketone extract of *Arthrinium phaeospermum*, MF 5629, gave a positive response in the ChTX binding assay.[6] Bio-assay guided purification and spectroscopic studies led to the identification of **1** (CAF603) as the active component.



Maxikdiol



1 R=H
2 R=$COCH_3$

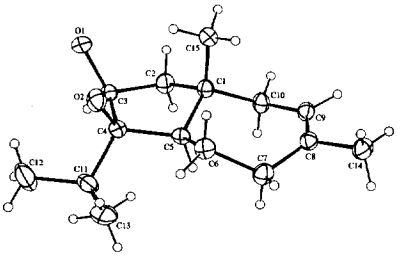


Figure 1. ORTEP drawing of **1**.

The dried extract of *A. phaeospermum* was solublized in ethyl acetate/methylene chloride mixture (1:1). Sequential chromatography of the soluble portion over silica gel, Sephadex® LH-20, a second silica gel step and finally reverse phase preparative HPLC yielded **1** (50 mg from 2.5 L) as a white powder.

^1H and ^{13}C NMR spectral data ($CDCl_3$), $[\alpha]_D$ -22.5°(C=0.55, MeOH) and mass spectrometric results (HR- EIMS, M^+238, $C_{15}H_{26}O_2$) of **1** were almost identical with the data reported for CAF-603, isolated by Wanatabe et.al. from *Trichoderma virens* (*Gliocladium virens*, IFO 9166).[7,8] A number of deviations in the NMR spectra from the reported values were observed: C-3 and C-4 hydroxyls at δ2.08 and at δ2.42 respectively were not observed; carbon signals reported at δ84.8 (C-4) and δ139.5 (C-8) differ by 0.4 ppm; H-3 of the monoacetate (**2**) was observed at δ5.0 ppm as opposed to δ4.0 ppm described by Wanatabe et.al. This prompted us to verify the structure by X-ray crystallography.

Compound **1** was crystallized from hexane as needles; X-ray studies confirmed the structure as proposed by Wanatabe et. al. and show both hydroxyls and the methyl group at C-1 to be on the same side of the 5-membered ring (Figure 1.). The atomic coordinates for the structure have been deposited with the Cambridge Crystallographic Data Centre. The absolute configuration could not determined without a heavy atom.

T. virens (ATCC 9645 = IFO 9166, IFO 6355) was fermented in several media.[9] This culture produced a compound which co-chromatographed with **1** . Furthermore, the compound isolated from ATCC 9645 had identical ^1H and ^{13}C NMR to that of **1.** Therefore, the inconsistencies noted above in the ^1H and ^{13}C NMR may be due to either typographical errors or concentration effects.

The concentration of **1** needed to produce 50% inhibition of [^{125}I]ChTX binding in aortic sarcolemmal membranes was 200 nM. Maximal inhibition observed was 90%. Compound **1** had no effect on [^{125}I]ChTX binding to voltage-dependent K^+ channels in rat brain synaptic plasma membranes.[10] Partial inhibition of [^{125}I]ChTX in smooth muscle membranes is an indicator that **1** is an allosteric modulator of toxin binding; suggesting that the MAXI-K channel, like other ion channels, is a multi-drug receptor. The biological profile of **1** is similar to that of maxikdiol (IC_{50} 1μM).[5] The functional activity of CAF-603 and other novel CAF-603 like sesquiterpenoids will be reported on elsewhere.[11]

Acknowledgments
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References and Notes

1. Latorre, R.; Vergara, C.; Hidalgo, C. *Proc. Natl. Acad. Sci.* **1981**, *293*, 471.
2. Stretton, D.; Motohiko, M.; Beluesi, M. G.; Barnea, P. J. *Proc. Natl. Acad. Sci.* **1992**, *89*, 1325.
3. Garcia, M. L.; Galvez, A.; Garcia-Calvo, M.; King, V.F.; Vasquez, J.; Kaczorowski, G.J. *J. Bioenrg. Biomembr.* **1991**, *23*,615.
4. Vazquez, J.; Feigenbaum, P.; Katz, G.; King, V.F.; Reuben, J.P.; Roy-Contncin, L.; Slaughter, R.S.; Kaczorowski,G. J.; Garcia, M. L. *J. Biol. Chem.* **1989**, *264*, 20902.
5. Singh, S. B.; Goetz, M. A.; Zink, D. L.; Dombrowski, A. W.; Polishook, J. D.; Garcia, M. L.; Schmalhofer, W.; McManus, O. B.; Kaczorowski, G. J. *J. Chem. Soc. Perkin Trans. 1*, **1994**, 3349.
6. This fungus (#3041) was received from MYCOsearch, Chapel Hill, North Carolina as an unidentified strain from twigs found in the Panama Canal Zone. At Merck, the strain was determined to be the ubiquitous epiphytic hyphomycete, *Arthrinium phaeospermum*, and was accessioned to the Merck Microbial Resources Culture Collection as MF 5629.
7. Watanabe, N.; Yamagishi, M.; Mizutani, T.; Kondoh, H.; Hanada, K.; Kushida, K. *J. Nat. Prod.* **1990**, *53*, 1176.
8. *Trichoderma virens* replaces *Gliocladium virens* mentioned in Wanatabe et. el.
9. Production of CAF-603 by ATCC 9645 was in casitone (1%) plus glucose (4%), 50 ml/250 ml flask, at 25°C, 220 rpm for 6 days. MF 5629 media: corn meal (5%), yeast extract (0.1%) and glucose (4%).
10. Vasquez, P.; Feigenbaum, P.; King, V. F.; Kaczorowski, G. J.; Garcia, M. L. *J. Biol Chem.* **1990**, *265*, 15564.
11. Lee, S. H.; Hensens, O. D.; Helms, G. L.; Liesch, J. M.; Zink, D. L.; Giacobbe, R. A.; Bills, G. F.; Stevens-Miles, S.; Garcia, M. L.; Schmalhofer, W. A.; McManus, O. B.; Kaczorowski, G. J. *J. Antibiotics* (submitted).

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