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Potent *Nor*-triterpenoid Blockers of the Voltage-gated Potassium Channel Kv1.3 from *Spachea correae*

Michael A. Goetz*, Otto D. Hensens*, Deborah L. Zink, Robert P. Borris, Francisco Morales^, Giselle Tamayo-Castillo^, Robert S. Slaughter, John Felix and Richard G. Ball

Merck Research Laboratories, P. O. Box 2000, Rahway, NJ 07065-0900 (USA)
and ^ Instituto Nacional de Biodiversidad, Heredia, Costa Rica

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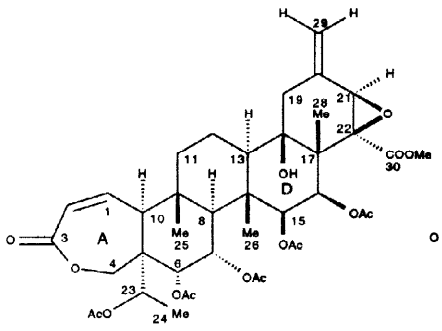
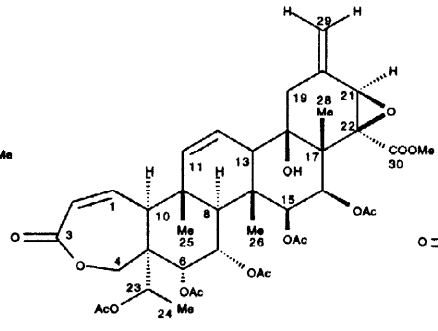
Abstract: *The isolation and structure elucidation of two novel nor-triterpenoid KV1.3 potassium channel blockers correolide and dehydrocorreolide from the Costa Rican tree Spachea correae are reported.*

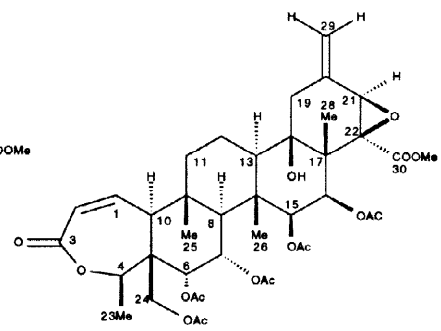
Activation of T lymphocytes *via* the antigen receptor results in a rise in the intracellular calcium required for T cell proliferation and clonal expansion. Block of mitogen-induced calcium increases leads to a block of IL-2 synthesis and release, and, ultimately, antiproliferative effects.[1] The voltage-gated delayed rectifier potassium channel KV1.3 appears to control the membrane potential in human T cells. Because the rise in intracellular calcium is dependent on membrane potential, agents that inhibit the opening of KV1.3 can be expected to have immunosuppressive properties. This effect has been demonstrated with a number of non-selective potassium channel blockers (tetraethylammonium, 4-aminopyridine) as well as with peptide toxins (charybdotoxin, noxiustoxin, margatoxin).[2,3]



1 correolide



2 dehydrocorreolide



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We report here on the identification, from extracts of the Costa Rican tree *Spachea correae* Cuatrec. & Croat (Malpighiaceae), of the first potent and selective small molecule blockers of KV1.3, correolide (**1**) and its dehydro derivative **2**. These are highly functionalized *nor*-triterpenoids possessing an unusual α,β-unsaturated-7-membered lactone A ring and containing a rather extraordinary number of acetyl groups. Compounds **1** and **2** were shown to depolarize T cells, thus stopping the calcium-dependent activation cascade and resulting in immunosuppression.[4]

An ethanol extract of the roots of *S. correae* was fractionated by chromatography on silica gel (methylene

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chloride containing increasing amounts of ethyl acetate) followed by gel filtration on Sephadex LH-20 to afford a mixture of **1** and **2**. These could be readily resolved by reverse phase HPLC using acetonitrile-water mixtures. Yield **1** : 2.3 mg/g root; **2** : 0.70 mg/g root. Purification on larger scale was more conveniently achieved by repeated column chromatography on silica gel to separate 1^5 and 2^5, followed by crystallization from methanol.

Table 1. ^1H and ^{13}C NMR Assignments of correolide (**1**) and dehydrocorreolide (**2**) (CD_2Cl_2, 500 MHz)[a]

	1		**2**	
Carbon	δ_C	δ_H	δ_C	δ_H
1	143.0	6.36 dd (8.7, 12.2)	143.3	6.48 dd (8.7, 12.2)
2	123.7	6.04 d (12.2)	124.3	6.11 d (12.2)
3	169.3 v.br		168.6	
4	66.7 v.br	4.44 br.d (~12.1)	67.8	3.89 d (12.3)
		4.09 d (12.5)		4.32 d (12.3)
5	49.2 br		50.4	
6	68.4 v.br	5.60 br. m	66.2	5.72 d (5.5)
6-OCOMe	170.3*/21.1*	2.00 s	169.5/21.7**	1.97 s
7	69.9 d	5.64 dd (3.8, 5.6)	68.6	5.54 dd (5.5,9)
7-OCOMe	170.8*/21.8*	1.96 s	171.7/20.8**	1.97 s
8	62.6	1.71 m	57.8	2.39 d (8.9)
9	40.8 v.br		42.3	
10	59.1	2.41 d (8.8)	54.0	2.81 d (8.7)
11	43.6 br	1.56 br.m (β)	137.1	5.59 dd (2.8, 10.8)
12	16.8	~1.71 m (2H)	121.1	5.67 dd (1.9, 10.8)
13	47.5	1.34 dd (2.9, 12.1)	48.1	2.39 t (~2.5)
14	42.1		40.6	
15	75.6	4.75 d (3.9)	73.8	4.94 d (3.9)
15-OCOMe	171.3/22.2	1.79 s	171.0/21.8	1.80 s
16	73.9	5.68 d (3.9)	116.7	7.70 s
16-OCOMe	169.2/20.9	2.10 s	169.3/20.9	2.09 s
17	45.1		45.4	
18	75.0		74.4	
19	35.6	2.24 dt (14.7, 1.9) (β)	35.1	2.34 dt (14.7, 1.5)
		2.13 br.d (14.7) (α)		2.24 br.d (14.7)
20	138.9		138.9	
21	63.5	4.00 s	63.7	3.87 br.s
22	66.1 v.br		66.1	
23	77.1 br	5.34 br.m	71.4	5.33 br.q (6.6)
23-OCOMe	170.2*/21.6*	2.07 br.s	169.9/21.9	2.14 s
24	17.1	1.23 br.d (6)	17.3	1.17 d (6.5)
25	20.7	1.05 br.s	21.3	1.13 s
26	15.2	1.46	14.9	1.41 s
28	15.0	1.27 s	14.8	1.32 s
29	119.4	5.47 br.s	119.5	5.23 br.s
		5.24 br.s		5.42 br.s
30	167.7		167.6	
30-OMe	53.5	3.91 s	53.5	3.91 s

[a] at 25°C; coupling constants are given in Hz in parentheses. Abbreviations: br = broad, v = very

* and ** interchangeable resonances.

High resolution EI-MS of correolide indicated the molecular formula $C_{40}H_{52}O_{16}$ (found m/z 788.3220; calcd m/z 788.3255) which was supported by the carbon and carbon-bound proton counts from ^{13}C NMR and

DEPT spectra (Table 1), respectively, assuming one exchangeable proton. However, the compound did not silylate (tertiary alcohol). Several of the ^{1}H and ^{13}C resonances were exchange-broadened as indicated in the Table. Structure **1** was established on the basis of 2D NMR and mass spectral comparison with its dehydro derivative **2** which in turn was determined by spectral and X-ray analysis (see below). The structure contains five acetate groups two of which are lost in the EI-MS as acetic acid from the molecular ion observed at m/z 728.3040 ($C_{38}H_{48}O_{14}$ calc. 728.3036) and m/z 668.2834 ($C_{36}H_{44}O_{12}$ calc. m/z 668.2836). The base peak at m/z 334.1417 ($C_{18}H_{22}O_6$, calcd m/z 334.1418) corresponds to both halves of the molecule resulting from ring C cleavage and loss of acetic acid. The five acetates and the unusual α,β-unsaturated-7-membered lactone A ring are reminiscent of the *nor*-triterpenoid **3** isolated from the Brazilian Amazonian plant *Lophanthera lactescens* Ducke.[6] The structures differ in ring A where lactone closure is through the 5-CH_2OH group and not the methyl carbinol hydroxyl group attached to C5.

The different ring closure was evident from the HMBC correlations from the C4 methylene protons to the lactone carbonyl carbon C3 (Figure 1). This feature was firmly established by X-ray on **2**[7] which also defined the relative stereochemistry indicating a *cis* instead of a *trans* A/B ring junction. Comparison of the NMR data in deuterochloroform tentatively suggested identity between the two structures therefore requiring modification of **3** to **1**. If the absolute configuration of the molecule is assumed to be correct then C23 has the *R* configuration.

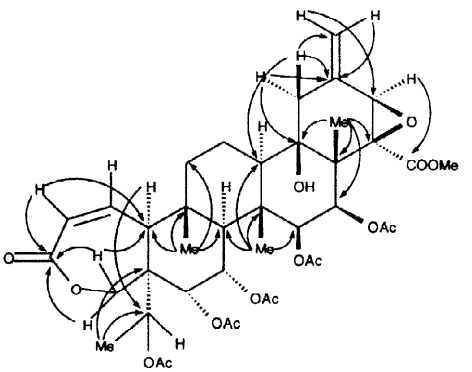

Figure 1. Selected HMBC correlations of correolide

Dehydrocorreolide was shown to have the molecular weight 786 by HR-EI corresponding to $C_{40}H_{50}O_{16}$, two protons less than the major compound **1** (found m/z 786.3075, calcd m/z 786.3051). Three successive losses of acetic acid from the molecular ion are observed. The molecular formula was supported by the carbon and carbon-bound proton counts from ^{13}C NMR and DEPT spectra (Table 1), respectively, which by contrast to **1** showed sharp spectra with all resonances clearly visible. Structure **2** was assigned on the basis of NMR ($^{1}H/^{13}C$, DEPT, COSY, HMQC and HMBC), MS and X-ray analysis.[7] The additional double bond in ring C changed the EI-MS fragmentation now giving the base peak at m/z 489.2099 (calcd m/z 489.2124). In this case a similar cleavage of ring B through bonds C6-C7 and C9-C10 was observed with loss of a molecule of water. Comparison of the NMR data for **1** and **2** are given in Table 1. The relative stereochemistry followed from analysis of the vicinal $^{3}J_{HH}$ couplings and NOESY data as depicted in Figure 2 and confirmed by the X-ray data where ring B predominantly adopts a twist-boat conformation with the *cis* A/B ring junction.

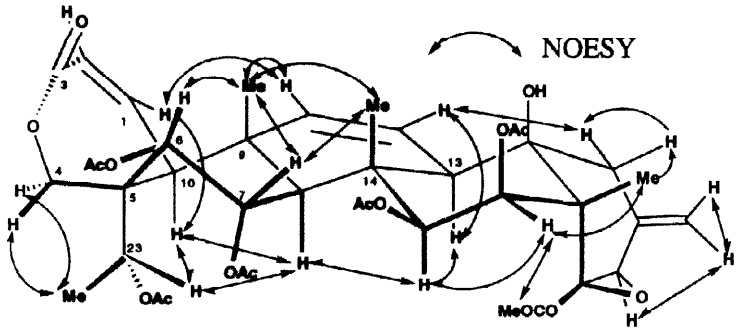

Figure 2. Relative stereochemistry and predominant conformation of dehydrocorreolide (**2**)

Acknowledgements

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References and Notes

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5. **1** m.p. 287-290° $[\alpha]_D^{22}$ +10.8° (c, 0.24, $CHCl_3$); **2** m.p. 299-302° $[\alpha]_D^{22}$ +8.6° (c, 0.31, $CHCl_3$).
6. Abreu, H. D., Fo, R. B.; Gottlieb, H. E.; and Shoolery, J. N. *Phytochemistry* **1990**, *29*, 2257-2261.
7. Crystal structure details for **2**: $C_{40}H_{50}O_{16} \cdot H_2O$, M_r = 804.850, orthorhombic, $P2_12_12_1$, a = 15.329(2), b = 19.718(1), c = 13.418(2) Å,, V = 4056(1) $Å^3$, Z = 4, D_x = 1.318 g cm^{-3}, monochromatized radiation $l(K_a)$ = 1.541838 Å, m =0.83 mm^{-1}, $F(000)$ = 1712, T = 294 K. Data collected on a Rigaku AFC5 diffractometer to a q limit of 70° with 1532 observed, at I 3s(I), reflections out of 4293 measured. Structure solved by direct methods and refined using full-matrix least-squares on F using 233 parameters. Compound **2** crystallizes with one molecule of water in the lattice and this water is disordered over two sites. The non-hydrogen atoms were refined with a mixture of isotropic and anisotropic thermal displacements and the hydrogen atoms were included at their calculated positions. Final agreement statistics are: R = 0.082, wR = 0.071, S = 2.42, $(D/s)_{max}$ = 0.01. Weighting scheme is $1/s^2(F)$. Maximum peak height in final difference Fourier map is 0.42(8) $eÅ^{-3}$. The authors have deposited the atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained on request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.