A biosynthetically inspired synthesis of (−)-berkelic acid and analogs

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ABSTRACT

We describe a complete account of our total synthesis and biological evaluation of (−)-berkelic acid and analogs. We delineate a synthetic strategy inspired by a potentially biomimetic union between the natural products spicifernin and pulvilloric acid. After defining optimal parameters, we executed a one-pot silver-mediated in situ dehydration of an isochroman lactol to methyl pulvillorate, the cyclo-isomerization of a spicifernin-like alkynol to the corresponding exocyclic enol ether, and a subsequent cycloaddition to deliver the tetracyclic core of berkelic acid. Our studies confirm that the original assigned berkelic acid structure is not stable and equilibrates into a mixture of 4 diastereomers, fully characterized by X-ray crystallography. In addition to berkelic acid, C22-*epi*-berkelic acid, and *nor*-berkelic acids, we synthesized C26-oxoberkelic acid analogs that were evaluated against human cancer cell lines. In contrast to data reported for natural berkelic acid, our synthetic material and analogs were found to be devoid of activity.

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1. Introduction

In 2006 Stierle et al. disclosed the tetracyclic chroman/iso-chroman/spiroketal natural product (−)-berkelic acid (Fig. 1) isolated from the fermentation broth of an extremophilic *Penicillium* fungus encountered in the Berkeley Pit Lake in Butte, Montana.[1] The Berkeley Pit Lake, which formed by groundwater seepage into an abandoned copper pit mine, is currently the United States' largest superfund cleanup site containing approximately 30 billion gallons of highly acidic (pH 2.5), heavy metal-contaminated (arsenic, copper, cadmium, cobalt, iron, manganese, and zinc) water.[2] Berkelic acid was found to be a moderate inhibitor of MMP-3 (1.87 μM) and the cysteine protease caspase-1 (98 μM), and, in testing against the NCI 60 cell line panel, was reported to possess selective activity against the human ovarian cancer cell line OVCAR-3 (GI_{50} 91 nM). However, a subsequent analysis of fully synthetic (−)-berkelic acid from the Snider group in the NCI 60 cell line panel indicated no activity against any of the cell lines (up to 10 μM) including OVCAR-3.[3]

The initially assigned structure of (−)-berkelic acid (**1**) was determined by NMR experiments, but did not address the configuration of the C22-quaternary stereocenter or the overall absolute configuration. Subsequent work of the Fürstner group led to a revised structure of berkelic acid (**2**) through an elegant synthetic, NMR, and crystallographic study culminating initially in the synthesis of both C22-epimers of the corresponding methyl esters of *ent*-**2**,[4] and subsequently (−)-berkelic acid (**2**).[5] These efforts established the absolute configuration of five stereocenters and a revision of the configuration at C18 and C19 in the original assigned structure. In fact, Fürstner noticed that an advanced tetracyclic intermediate with configuration reminiscent of the original structure could not be prepared as a single diastereomer. An unfavorable eclipsing interaction between C16 and C25 instead provided a driving force for equilibration into a nearly statistical mixture of four diastereomers epimeric at C15, C17, and C18.[6,7] Unfortunately, insufficient spectroscopic differences between two synthetic methyl berkelates epimeric at C22, lack of an authentic sample, and the inability to selectively saponify the C1-methyl benzoate prevented an unambiguous assignment of the C22 stereocenter. Later, Snider and coworkers reported the first total synthesis of berkelic acid, confirming Fürstner's structural revision and putatively assigning the quaternary stereocenter as C22-*S*.[3,8] As the C22 stereocenter was introduced via a non-selective Kiyooka aldol reaction,[9] the stereochemical assignment relied upon correlation of the resultant diastereomers to a model compound. Our subsequent

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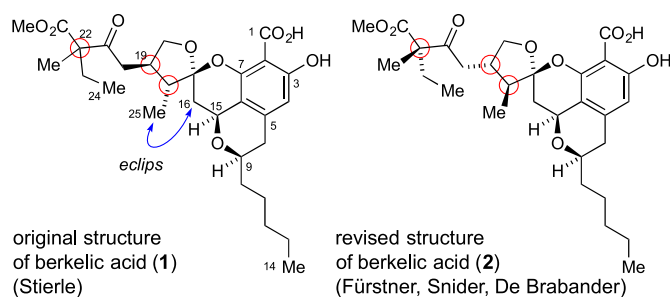*E-mail address:* Jef.DeBrabander@UTSouthwestern.edu (J.K. De Brabander).

original structure
of berkelic acid (**1**)
(Stierle)

revised structure
of berkelic acid (**2**)
(Fürstner, Snider, De Brabander)

Fig. 1. Original and revised structures of berkelic acid.

synthesis of both C22-epimers of berkelic acid fully corroborated the Snider assignment.[10] Finally, a gram-scale total synthesis was reported in 2012 by Fañanás and Rodríguez that centered on a silver-catalyzed addition/cyclization cascade of appropriately substituted alkyne and aldehyde precursors to set four new chiral centers in a 2:1 diastereomeric ratio.[11–13]

Constitutionally, the originally proposed structure of (−)-ber-kelic acid (**1**) emerges as an amalgamation of two other natural products, namely pulvilloric acid (**4**)[14] and spicifernin (**3**). The co-isolation of berkelic acid with spiciferone A (**5**),[1] which previously had been isolated alongside spicifernin (**3**) from a *Cochliobolus* fungus,[15] is indeed suggestive of a proposal that berkelic acid could be the end-product of such a biosynthetic merger (Scheme 1). Biosynthetic studies utilizing [13]C- and [2]H-labeling have specified that both spicifernin (**3**) and spiciferone A (**5**) are generated from a common hexaketide precursor.[15,16] Thus, the presence of spicifer-one A (**5**) in the berkelic acid culture medium suggests that the Berkeley Pit Lake *Penicillium* sp. might also possess the biosynthetic machinery to produce spicifernin (**3**). To account for the stereo-chemical discrepancy between natural spicifernin (**3**) and (−)-berkelic acid (**2**), which display an epimeric relationship at C18 and C19 (berkelic acid numbering), Snider proposed that spic-ifernin (**3**) or an immediate biosynthetic precursor could undergo a double epimerization (C18 and C19) followed by a reduction to provide a correctly configured bis-*epi*-deoxyspicifernin (**6**) for addition to pulvilloric acid (**4**).[3,17] We proposed an alternative hy-pothesis where spicifernin (**3**) would be unified with pulvilloric acid (**4**) first, perhaps via its dehydrated enollactone **7** in a formal cycloaddition with the ortho-quinone methide tautomer of pul-villoric acid, followed by a double epimerization (at C18 and C19) and final reduction.[18,19]

Given berkelic acid's unique structural features, stereochemical questions, biological activity, and potential biosynthetic origin from combination of two other natural products, we embarked on a synthetic program that culminated in the total synthesis of (−)-berkelic acid (**2**) communicated several years ago.[10] Here, we present a full account of our extensive research program including the development of a novel silver-catalyzed dehydration/cyclo-isomerization/cycloaddition cascade to forge the tetracyclic chro-man/isochroman/spiroketal structure embedded within berkelic acid, the synthesis and full characterization of diastereomers of the originally proposed structure, C22-geminal dimethyl substituted analogs, and C26-oxoberkelates to explore the proposed biosyn-thesis, and biological evaluation of synthetic berkelic acid and analogs.

2. Results and discussion

Initially, we became interested in a synthetic campaign toward berkelic acid due to a unique constellation of structural features that we perceived as a fruitful platform to implement methodology developed in our group. In our initial decisively non-biomimetic analysis, we thus perceived linear polyhydroxy alkyne **9** as a suit-able direct precursor to berkelic acid (**2**) if conditions could be identified to forge a one-pot cycloisomerization/dehydrative cycloetherification as depicted in Scheme 2. The dividend pay-off would be even higher if thus identified reaction conditions could operate on a protecting group free substrate with all final func-tionality at the correct oxidation state (i.e. substrate **9**). Based on studies from our group, we had considerable confidence in executing such a strategy. Indeed, as shown in the box in Scheme 2, we developed methodology to exploit internal alkynes as a po-tential nucleus for metal-catalyzed remolding of linear substrates to cyclic/polycyclic structures. In 2006, we reported on the metal-catalyzed cycloisomerization of dihydroxy-alkynes to form spi-roketals under mild conditions (relevant to path a, **10** → **11**)[20] fol-lowed by propargylic cycloetherification in 2008 (relevant to transformation **10** → **12**)[21] and a tandem combination that would yield **13** directly from **10** (via **12**; propargylic substitution preceding cycloisomerization).[22] En route to berkelic acid, we envisioned polyhydroxy-alkyne precursor **9** to be available from the addition of terminal alkyne **14** to aldehyde **15** (C15-C16 bond formation). However, if we were to implement a protecting group free syn-thesis, this aldehyde **15** would entirely exist as the corresponding non-electrophilic lactol **16**, leading us to contemplate the natural product pulvilloric acid (**4**, the dehydration product of **16**) as a viable electrophilic coupling partner instead. It is at this point that our synthetic analysis metamorphosed to one that appeared more reminiscent of the biosynthetic pathway proposed in Scheme 1 and contemplated that a metal-catalyzed 5-*exo*-dig hydroalkoxylation of alkynol **14** should deliver enol **17**,[20] a material that would combine with the ortho-quinone methide tautomer of pulvilloric acid (**4**) via a formal cycloaddition to deliver directly the chroman/isochroman/spiroketal tetracycle of berkelic acid (i.e. bypassing proposed precursor **9**). Ideally, a one-pot operation would be identified that enables (1) the dehydration of lactol **16** to pulvilloric acid **4**, (2) the cycloisomerization of **14** to **17**, and (3) the final cycloaddition between **4** and **17** to deliver berkelic acid (**2**).[23]

Our synthetic studies began with the development of a concise route to a lactol similar to **16** as a precursor to pulvilloric acid (**4**). As shown in Scheme 3, selective triflation of commercially available methyl 2,4,6-trihydroxybenzoate **18** provided triflate **19**, which readily underwent Suzuki-Miyaura cross coupling with known (E)-1-heptenylboronic acid (**20**)[24] to yield styrene derivative **21** in 83% yield from commercial **18**.[25] Installation of the homobenzylic alcohol was best accomplished via a three-step sequence from **21** including MOM protection of the free phenols, styrene epoxidation with m-CPBA, and benzylic hydrogenolysis providing alcohol **22** in 76% overall yield from **21**.[26] Methanolysis of the MOM protecting groups and methyl ester delivered alcohol **23** in 97% yield and was followed by a condensation with triethyl orthoformate via a pro-cedure adapted from the synthesis of pulvilloric acid[14c] to deliver the ethanol adduct of methyl pulvillorate **24** in excellent yield.

With a viable racemic route established, we next explored the possibility to intercept racemic homobenzylic alcohol **22** for a late-stage enzymatic resolution. We subjected (±)-**22** to a set of 21 different lipases for acetylation of the homobenzylic alcohol with vinyl acetate and identified three enzymes that provided the desired acetate **25** at a synthetically practical rate. Of these, a lyophilized formulation of lipase from *Alcaligenes* sp. exhibited high selectivity and scalability, allowing for the preparation of (R)-**25** and (S)-**22** in 95% and 93% ee, respectively.[27] As it was ultimately discovered that berkelic acid was of the C9-(R) configuration,[3–5] a Mitsunobu esterification of the enantioenriched homobenzylic alcohol (S)-**22** (93% ee) with acetic acid provided additional (R)-**25** in 78% yield, for an overall yield of 85% of (R)-**25** from (±)-**22**. Global